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**(54) Neuronal cell-specific receptor protein**

(57) To provide a method of isolating and detecting a new receptor gene, as a means of elucidating the function of neuronal cell-specific receptors, especially of elucidating the detailed mechanism of the neuronal cell differentiation inhibitory and nerve nutrition factor-like actions of activin receptors, DNA containing said new receptor gene, a method of producing a protein encoded by said new receptor gene, and use for said DNA and said protein. The receptor protein of the present invention and DNA encoding said protein can be used for various purposes, including 1) ligand determination, 2) obtainment of antibodies and antisera, 3) construction of recombinant receptor protein expression systems, 4) development of receptor binding assay systems, 5) screening for pharmaceutical candidate compounds using such expression systems, 6) drug designing based on comparison with structurally similar ligand receptors, 7) reagent for preparation of probes and PCR primers for gene diagnosis, and 7) drug for gene therapy.

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**Descripti n**Technical Field

5 The present invention relates to a receptor protein having a particular amino acid sequence, particularly a new neuronal cell-specific receptor, more particularly a new activin receptor protein, DNA containing a DNA region encoding said protein, a production method for said protein, a detection method for the gene encoding said protein, and use for said protein and DNA.

10 Background Art

To date, numerous bioactive substances exhibiting diverse actions on various organs and cells have been isolated and identified, and their functions elucidated. The diverse bioactivity of these substances in various organs and cells is normally expressed via receptors to which they are bound; however, it remains unknown as to whether all organs and cells share the same receptors or if the organ and cell specificity of receptors is variable.

15 Steroid hormones produced by reproductive glands were assumed to be responsible for the regulation of gonadotropic hormone secretion from the pituitary gland. Then activin was first isolated as a regulator that promotes the secretion of follicle-stimulating hormone (FSH) from the anterior lobe of the pituitary gland. After the discovery of activin and inhibin, which antagonizes activin, activin and inhibin have drawn attention as facilitating a new mechanism of hormone secretion regulation for the hypothalamus-pituitary-reproductive organ system. Analysis of the bioactivity of activin has revealed various biological actions, including induction or inhibition of cell differentiation in the blood cell system and reproductive organs, and neuronal cell survival maintenance action, in addition to regulation of FSH secretion. However, much remains unknown as to activin's detailed mechanism of action.

20 The activin receptor gene has been cloned in humans, mice, rats and other animals and structural analysis has revealed two types: types I and II. It has also been shown that types I and II each have two subtypes, A and B, and that type IIB can be divided into four subtypes by alternative splicing. Receptor proteins assumed to mediate the diverse bioactivity of activin have varying affinity for different forms of activin; their expression is known to occur over a wide range without organ or cell specificity. Activin is known to induce or inhibit cell differentiation, but the expression of activin or its receptors has not been known to significantly vary during all differentiation. The mechanism of diverse actions of

30 activin remains to be elucidated in detail.

Summary of the Invention

The object of the present invention is to develop a method for elucidating the function of receptors, especially neuronal cell-specific receptors, more specifically to provide an isolation and detection method for a new nerve system-specific activin receptor gene, DNA containing said new activin receptor gene, a production method for a protein encoded by said new activin receptor gene, and use for said DNA and said protein, as a tool for elucidating the detailed mechanism of neuronal cell differentiation inhibition and nerve nutrition factor-like action of activin receptors.

40 With the aim of solving the above problems, the present inventors made extensive investigation, and succeeded in amplifying a cDNA fragment encoding activin receptor type IIA protein from poly(A)<sup>+</sup> RNA from P19, a mouse embryonal carcinoma cell line whose neuronal differentiation was induced with retinoic acid by reverse transcriptase polymerase chain reaction; (hereinafter referred to as the RT-PCR method) using synthetic DNA primers for efficient isolation of cDNA encoding activin receptor type IIA protein. As a result of analysis of the cDNA fragment, the present inventors isolated DNA encoding a new receptor protein. Further analysis of the base sequence of the DNA demonstrated that

45 said DNA encodes a new receptor protein containing eight amino acids shown by SEQ ID NO:7. The present inventors made further investigation, and found that said receptor gene is specifically expressed in neuronal cells and organs.

The present inventors also found that the expression of the known activin receptor type IIA gene and that of said new receptor gene can easily be differentiated and separately detected by the RT-PCR method using said synthetic DNA primers.

50 More specifically, the present inventors obtained cDNA fragments from poly(A)<sup>+</sup> RNA from P19, a mouse embryonal carcinoma cell line whose neuronal differentiation was induced with retinoic acid, by the RT-PCR method using as primers the two synthetic DNAs shown by SEQ ID NO:1 and SEQ ID NO:2 selected from the known mouse activin receptor type IIA gene shown in Figures 1 and 2 (M65287, GenBank data registration number), resulting in the amplification of a longer cDNA fragment, together with a fragment having the same length as that of the fragment deduced from the known base sequence (nucleotide sequence). This longer cDNA fragment was subjected to base sequence analysis. The present inventors found that these two cDNAs respectively encode the known activin receptor type IIA gene (M65287) and a new receptor wherein the 24 bp sequence shown by SEQ ID NO:3 is inserted to the splicing site of the known activin receptor type IIA gene (M65287).

Next, the present inventors prepared cDNA from poly(A)<sup>+</sup> RNA from P19, a mouse embryonal carcinoma cell line

whose neuronal differentiation was induced with retinoic acid, and inserted it into  $\lambda$ ZAPII phage to yield a cDNA library. The present inventors further screened this library using as a probe the cDNA having a 24 bp sequence which was inserted to the splicing site of the known activin receptor type IIA gene (M65287), obtained by the RT-PCR method, and obtained a DNA fragment encoding the entire new receptor protein of the present invention. This DNA fragment was subjected to base sequence analysis, resulting in the finding that its base sequence was identical to that of the activin receptor type IIA gene (M65287), except for the 24 bp insertion discovered by the present inventors, as shown by SEQ ID NO:4, and that the amino acid sequence deduced therefrom is shown by SEQ ID NO:5 (see Figures 3 through 6).

The present inventors further extracted poly(A)<sup>+</sup> RNA from various mouse organs, and examined the expression of the present new receptor by the RT-PCR method using as primers the synthetic DNAs shown by SEQ ID NO:1 and SEQ ID NO:2, resulting in the finding that its expression is specific to the neuronal system (e.g. brain) and the whole embryo, as shown in Figure 7.

In addition, the present inventors obtained cDNA fragments by the RT-PCR method using as primers the synthetic DNAs shown by SEQ ID NO:1 and SEQ ID NO:2 from poly(A)<sup>+</sup> RNA from a human neuroblastoma cell line, and obtained cDNA fragments by the RT-PCR method using as primers the synthetic DNAs shown by SEQ ID NO:9 and SEQ ID NO:10 from poly(A)<sup>+</sup> RNA from frog embryo, and found that longer cDNA fragments were amplified, together with those of the same length as that of the fragment deduced from the known base sequence, as described above. These cDNA fragments were subjected to base sequence analysis, resulting in the finding that the same 24 bp sequence was inserted both in humans and frogs, and that the base sequence in humans is identical to that in mice, while the base sequence in frogs is the base sequence shown by SEQ ID NO:6. The present inventors also found that these base sequences shown by SEQ ID NO:3 and SEQ ID NO:6, when translated to amino acid sequences, correspond to the amino acid sequence shown by SEQ ID NO:7, i.e., the amino acid sequence is preserved to very high degree without species specificity.

Accordingly, the present invention provides:

(1) A receptor protein containing an amino acid sequence substantially shown by the formula:

His Ala Phe His Ile Met Ile Glu

or a salt thereof,

(2) the protein described in the above item (1) wherein said receptor protein is a neuronal cell-specific receptor protein,

(3) the protein described in the above item (2) wherein said receptor protein is an activin receptor protein,

(4) a receptor protein containing or comprising an amino acid sequence shown by

X- Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln  
Glu Cys Leu Phe Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly Asp Lys Asp  
Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile  
Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu Lys Lys Asp Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys  
Asn Glu Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn  
Ile Leu Leu Tyr Ser Leu Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe Trp Val Tyr Arg His His Lys Met Ala  
Tyr Pro Pro Val Leu Val Pro Thr Gln His Ala Phe His Ile Met Ile Glu Asp Pro Gly Pro Pro Pro Ser Pro Leu Leu  
Gly Leu Lys Pro Leu Gln Leu Leu Glu Val Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln Leu Asn Glu  
Tyr Val Ala Val Lys Ile Phe Pro Ile Gln Asp Lys Gln Ser Trp Gln Asn Glu Tyr Glu Val Tyr Ser Leu Pro Gly Met Lys His  
Glu Asn Ile Leu Gln Phe Ile Gly Ala Glu Lys Arg Gly Thr Ser Val Asp Val Asp Leu Trp Leu Ile Thr Ala Phe His Glu  
Lys Gly Ser Leu Ser Asp Phe Leu Lys Ala Asn Val Thr Ser Trp Asn Glu Leu Cys His Ile Ala Glu Thr Met Ala Arg Gly  
Leu Ala Tyr Leu His Glu Asp Ile Pro Gly Leu Lys Asp Gly His Lys Pro Ala Ile Ser His Arg Asp Ile Lys Ser Lys Asn  
Val Leu Leu Lys Asn Asn Leu Thr Ala Cys Ile Ala Asp Phe Gly Leu Ala Leu Lys Phe Glu Ala Gly Lys Ser Ala Gly  
Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe  
Leu Arg Ile Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Ala Ser Arg Cys Thr Ala Ala Asp Gly Pro Val Asp Glu  
Tyr Met Leu Pro Phe Glu Glu Glu Ile Gly Gln His Pro Ser Leu Glu Asp Met Glu Glu Val Val Val His Lys Lys Lys Arg  
Pro Val Leu Arg Asp Tyr Trp Gln Lys His Ala Gly Met Ala Met Leu Cys Glu Thr Ile Glu Glu Cys Trp Asp His Asp Ala  
Glu Ala Arg Leu Ser Ala Gly Cys Val Gly Glu Arg Ile Thr Gln Met Gln Arg Leu Thr Asn Ile Ile Thr Thr Glu Asp Ile Val  
Thr Val Val Thr Met Val Thr Asn Val Asp Phe Pro Pro Lys Glu Ser Ser Leu,

wherein X represents Met that may be protected or hydrogen, or any portion thereof which also contains the amino acid sequence substantially shown by the formula: His Ala Phe His Ile Met Ile Glu, or a salt thereof,

(5) the protein described in the above item (3) having an amino acid sequence shown by

X- Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln  
Glu Cys Leu Phe Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly Asp Lys Asp  
Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile  
Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu Lys Lys Asp Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys

Asn Glu Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn  
 Ile Leu Leu Tyr Ser Leu Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe Trp Val Tyr Arg His His Lys Met Ala  
 Tyr Pro Pro Val Leu Val Pro Thr Gln His Ala Phe His Ile Met Ile Glu Asp Pro Gly Pro Pro Pro Pro Ser Pro Leu Leu  
 Gly Leu Lys Pro Leu Gln Leu Leu Glu Val Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln Leu Leu Asn Glu  
 5 Tyr Val Ala Val Lys Ile Phe Pro Ile Gln Asp Lys Gln Ser Trp Gln Asn Glu Tyr Glu Val Tyr Ser Leu Pro Gly Met Lys His  
 Glu Asn Ile Leu Gln Phe Ile Gly Ala Glu Lys Arg Gly Thr Ser Val Asp Val Asp Leu Trp Leu Ile Thr Ala Phe His Glu  
 Lys Gly Ser Leu Ser Asp Phe Leu Lys Ala Asn Val Val Ser Trp Asn Glu Leu Cys His Ile Ala Glu Thr Met Ala Arg Gly  
 Leu Ala Tyr Leu His Glu Asp Ile Pro Gly Leu Lys Asp Gly His Lys Pro Ala Ile Ser His Arg Asp Ile Lys Ser Lys Asn  
 Val Leu Leu Lys Asn Asn Leu Thr Ala Cys Ile Ala Asp Phe Gly Leu Ala Leu Lys Phe Glu Ala Gly Lys Ser Ala Gly  
 10 Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe  
 Leu Arg Ile Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Ala Ser Arg Cys Thr Ala Ala Asp Gly Pro Val Asp Glu  
 Tyr Met Leu Pro Phe Glu Glu Glu Ile Gly Gln His Pro Ser Leu Glu Asp Met Gln Glu Val Val Val His Lys Lys Lys Arg  
 Pro Val Leu Arg Asp Tyr Trp Gln Lys His Ala Gly Met Ala Met Leu Cys Glu Thr Ile Glu Glu Cys Trp Asp His Asp Ala  
 Glu Ala Arg Leu Ser Ala Gly Cys Val Gly Glu Arg Ile Thr Gln Met Gln Arg Leu Thr Asn Ile Ile Thr Thr Glu Asp Ile Val  
 15 Thr Val Val Thr Met Val Thr Asn Val Asp Phe Pro Pro Lys Glu Ser Ser Leu,

wherein X represents Met that may be protected or hydrogen; or a salt thereof,

(6) a protein containing an amino acid sequence substantially comprised of amino acid residues His Ala Phe His  
 20 Ile Met Ile Glu, wherein said protein is capable of binding with activin or activin like substances or a portion thereof,  
 (7) an isolated and purified DNA sequence encoding a protein containing an amino acid sequence sufficiently  
 duplicative of the sequence His Ala Phe His Ile Met Ile Glu to allow possession of the biological property of binding  
 with activin or activin containing substances,

(8) a DNA containing a nucleotide sequence encoding the protein described in the above item (1),

(9) the DNA described in the above item (8), containing a nucleotide sequence shown by

X' GGA GCT GCT GCA AAG TTG GCG TTC GCC GTC TTT CTT ATC TCT TGC TCT TCA GGT GCT ATA CTT  
 GGC AGA TCA GAA ACT CAG GAG TGT CTT TTC TTT AAT GCT AAT TGG GAA AGA GAC AGA ACC AAC CAG  
 ACT GGT GTT GAA CCT TGC TAT GGT GAT AAA GAT AAA CGG CGA CAT TGT TTT GCT ACC TGG AAG AAT  
 ATT TCT GGT TCC ATT GAA ATA GTG AAG CAA GGT TGT TGG CTG GAT GAT ATC AAC TGC TAT GAC AGG  
 30 ACT GAT TGT ATA GAA AAA AAA GAC AGC CCT GAA GTG TAC TTT TGT TGC TGT GAG GGC AAT ATG TGT  
 AAT GAA AAG TTC TCT TAT TTT CCG GAG ATG GAA GTC ACA CAG CCC ACT TCA AAT CCT GTT ACA CCG  
 AAG CCA CCC TAT TAC AAC ATT CTG CTG TAT TCC TTG GTA CCA CTA ATG TTA ATT GCA GGA ATT GTC  
 ATT TGT GCA TTT TGG GTG TAC AGA CAT CAC AAG ATG GCC TAC CCT CCT GTA CTT GTT CCT ACT CAA  
 CAC GCC TTT CAT ATA ATG ATA GAG GAC CCA GGA CCA CCC CCA CCT TCC CCA TTA CTA GGG TTG AAG  
 35 CCA TTG CAG CTG TTA GAA GTG AAA GCA AGG GGA AGA TTT GGT TGT GTC TGG AAA GCC CAG TTG  
 CTC AAT GAA TAT GTG GCT GTC AAA ATA TTT CCA ATA CAG GAC AAA CAG TCC TGG CAG AAT GAA TAT  
 GAA GTC TAT AGT CTA CCT GGA ATG AAG CAT GAG AAC ATA CTA CAG TTC ATT GGT GCA GAG AAA AGA  
 GGC ACC AGT GTG GAT GTG GAC CTG TGG CTA ATC ACA GCA TTT CAT GAA AAG GGC TCA CTG TCA  
 GAC TTT CTT AAG GCT AAT GTG GTC TCT TGG AAT GAA CTT TGT CAT ATT GCA GAA ACC ATG GCT AGA  
 40 GGA TTG GCA TAT TTA CAT GAG GAT ATA CTT GGC TTA AAA GAT GGC CAC AAG CCT GCA ATC TCT CAC  
 AGG GAC ATC AAA AGT AAA AAT GTG CTG TTG AAA AAC AAT CTG ACA GCT TGC ATT GCT GAC TTT GGG  
 TTG GCC TTA AAG TTC GAG GCT GGC AAG TCT GCA GGT GAC ACC CAT GGG CAG GTT GGT ACC CGG  
 AGG TAT ATG GCT CCA GAG GTG TTG GAG GGT GCT ATA AAC TTC CAA AGG GAC GCA TTT CTG AGG ATA  
 GAT ATG TAC GCC ATG GGA TTA GTC CTA TGG GAA TTG GCT TCT CGT TGC ACT GCT GCA GAT GGA CCC  
 45 GTA GAT GAG TAC ATG TTA CCA TTT GAG GAA GAA ATT GGC CAG CAT CCA TCT CTT GAA GAT ATG CAG  
 GAA GTT GTT GTG CAT AAA AAA AAG AGG CCT GTT TTA AGA GAT TAT TGG CAG AAA CAT GCA GGA ATG  
 GCA ATG CTC TGT GAA ACG ATA GAA GAA TGT TGG GAT CAT GAT GCA GAA GCC AGG TTA TCA GCT GGA  
 TGT GTA GGT GAA AGA ATT ACT CAG ATG CAA AGA CTA ACA AAT ATC ATT ACT ACA GAG GAC ATT GTA  
 ACA GTG GTC ACA ATG GTG ACA AAT GTT GAC TTT CCT CCC AAA GAA TCT AGT CTA,

50 wherein X' represents ATG or hydrogen,

(10) a vector containing the DNA described in the above item (8),

(11) a transformant harboring the vector described in the above item (10),

55 (12) a method of producing the protein described in the above item (1) by culturing the transformant described in  
 the above item (11) to produce and accumulate the protein or a salt thereof in the culture, and collecting said pro-  
 tein,

(13) a method of producing the protein described in the above item (1) by culturing the transformant described in  
 the above item (11) to produce and accumulate the protein or a salt thereof in the culture supernatant or on the

transformant's cell membrane, and collecting said protein,

(14) a method of detecting the DNA described in the above item (8) characterized by conducting the polymerase chain reaction method using synthetic DNA containing the base sequence of the formula:

TACCCTCCTGTACTTGTCTCTACTCAA

and the formula:

TAGCCACAGGTCCACATCCACACTGGT,

(15) an antibody against the protein described in the above item (1),

(16) a method of quantifying the protein described in the above item (1) by competitively reacting a subject solution containing the protein described in the above item (1) and the labeled protein in the above item (1) to the antibody in the above item (15),

(17) a method of determining a ligand against the protein described in the above item (1), wherein the protein described in the above item (1) is brought into contact with a test compound,

(18) a method of screening for a compound that inhibits or promotes the binding of the protein described in the above item (1) with a ligand wherein comparison is made between the binding affinities achieved in case (i) in which the ligand is brought into contact with the protein described in the above item (1), and case (ii) in which both the ligand and the compound are brought into contact with the protein described in the above item (1),

(19) a screening kit containing the protein described in the above item (1) and a ligand against the protein described in the above item (1), which screens for a compound that inhibits or promotes (i) the binding of a ligand against the protein described in the above item (1) or (ii) the binding of a ligand against a known activin receptor protein or a salt thereof,

(20) an activin receptor agonist or activin receptor antagonist obtained by using the screening method described in the above item (18) and/or the screening kit described in the above item (19),

(21) a diagnostic method for nerve degeneration disease wherein the DNA detection method described in the above item (14) is used.

#### Brief Description of the Drawings

Figure 1 shows the first half of the entire base sequence of cDNA encoding the mouse activin receptor type IIA (ATR) protein and the first half of the amino acid sequence deduced therefrom. Underlined portion (single line) indicates the position of the synthetic primer used for RT-PCR. (To be continued to Figure 2)

Figure 2 shows the last half of the entire base sequence of cDNA encoding the mouse activin receptor type IIA (ATR) protein and the last half of the amino acid sequence deduced therefrom. (Continued from Figure 1)

Figure 3 shows the first half of the entire base sequence of new receptor cDNA obtained from mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid by the RT-PCR method. (To be continued to Figure 4)

Figure 4 shows the last half of the entire base sequence of new receptor cDNA obtained from mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid by the RT-PCR method. (Continued from Figure 3)

Figure 5 shows the first half of the amino acid sequence deduced from the base sequence shown in Figures 3 and 4. (To be continued to Figure 6)

Figure 6 shows the last half of the amino acid sequence deduced from the base sequence shown in Figures 3 and 4. (Continued from Figure 5)

Figure 7 shows an analysis of expression by the RT-PCR method using poly(A)<sup>+</sup> RNA from various mouse organs or mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid. (See Example 5.) In the figure, ATR represents an RT-PCR product corresponding to known mouse activin receptor type IIA; ATR IIA-N represents an RT-PCR product corresponding to the new receptor of the present invention.

Figure 8 shows the first half of the entire base sequence of cDNA encoding frog activin receptor type II protein. (To be continued to Figure 9)

Figure 9 shows the last half of the entire base sequence of cDNA encoding frog activin receptor type II protein. (Continued from Figure 8)

#### Detailed Description of the Invention

The receptor protein, especially, the neuronal cell-specific receptor protein of the present invention may be any protein, as long as it contains the amino acid sequence substantially shown by SEQ ID NO:7, but preference is given to activin receptor protein. The amino acid sequence substantially shown by SEQ ID NO:7 possesses substantially the same activity as that of a protein containing the amino acid sequence shown by SEQ ID NO:7, and is exemplified by amino acid sequences resulting from deletion of 1 or more (preferably 1 or 2, more preferably 1) amino acid(s) from the

amino acid sequence shown by SEQ ID NO:7, amino acid sequences resulting from addition or insertion of 1 or more (preferably 1 or 2, more preferably 1) amino acid(s) to the amino acid sequence shown by SEQ ID NO:7, and amino acid sequences resulting from replacement with other amino acids of 1 or more (preferably 1 or 2, more preferably 1) amino acid(s) from the amino acid sequence shown by SEQ ID NO:7. Any receptor protein serves for the present invention, as long as it is derived from any tissue (e.g., stomach, pituitary, spleen, brain, kidney, liver, reproductive gland, thyroid gland, gallbladder, bone marrow, adrenal, skin, muscle, lung, digestive tract, blood vessel, heart) or cell of warm-blooded animals (e.g., guinea pigs, rats, mice, rabbits, pigs, sheep, bovines, monkeys, humans) and as long it contains the amino acid sequence substantially shown by SEQ ID NO:7, but preference is given to one having substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO:5. In other words, the receptor protein of the present invention may have any length, as long as it contains the amino acid sequence substantially shown by SEQ ID NO:7. Specifically, the receptor protein of the present invention may have a length of the peptide shown by SEQ ID NO:7, which is of the 8-amino acid sequence length, to a length of the amino acid sequence shown by SEQ ID NO:5, which is 521 amino acids in length, or longer. Preferable proteins include those that contain both the amino acid sequence substantially shown by SEQ ID NO:7 and an amino acid sequence possessing about 80-99.9% homology to the amino acid sequence shown by SEQ ID NO:5, and that possess substantially the same activity as that of a protein containing the amino acid sequence shown by SEQ ID NO:5, with greater preference given to proteins that contain the amino acid sequence substantially shown by SEQ ID NO:7, that has an amino acid sequence, without interruption, between the N- and C-ends of the amino acid sequence shown by SEQ ID NO:5 wherein one of the first amino acid (Met) through the 105th amino acid (Cys) of the 521-amino acid sequence shown by SEQ ID NO:5 constitutes the N-end and one of the 417th amino acid (Asp) through the 521st amino acid (Leu) constitutes the C-end, and that has an amino acid sequence length of not shorter than 417 amino acids. The first amino acid (Met) of the amino acid sequence shown by SEQ ID NO:5 may be protected or converted to hydrogen. Substantially the same activity is exemplified by ligand-binding activity and signal information transmission. Quantitative factors, such as ligand binding activity strength and receptor protein molecular weight may therefore differ.

More specifically, the receptor protein of the present invention (neuronal cell-specific receptor protein, more preferably activin receptor protein) is exemplified by the activin receptor protein from P19, a mouse embryonal carcinoma cell line, which contains the amino acid sequence shown by SEQ ID NO:5. The receptor protein of the present invention is also exemplified by amino acid sequences resulting from deletion of 1 or more (preferably 2 to 30, more preferably 2 to 10) amino acids from the amino acid sequence shown by SEQ ID NO:5 but other than SEQ ID NO:7, amino acid sequences resulting from addition or insertion of 1 or more (preferably 2 to 30, more preferably 2 to 10) amino acids to amino acid sequences other than SEQ ID NO:7 in SEQ ID NO:5, and amino acid sequences resulting from replacement with other amino acids of 1 or more (preferably 2 to 30, more preferably 2 to 10) amino acids from SEQ ID NO:5 but other than SEQ ID NO:7. The receptor protein of the present invention is still further exemplified by proteins wherein the N-terminal Met has been converted to H, those wherein the N-terminal Met is protected by a protecting group (e.g., C<sub>1-6</sub> acyl group such as formyl group or acetyl group, preferably C<sub>1-6</sub> alkanoyl group), those wherein the N-terminal side of Glu has been cleaved in the body, resulting in the formation of pyroglutamated Glu, those wherein the intramolecular amino acid side chain is protected by an appropriate protective group (e.g., C<sub>1-6</sub> acyl group such as formyl group or acetyl group, preferably C<sub>1-6</sub> alkanoyl group), and complex proteins such as glycoproteins resulting from binding of a sugar chain.

The salt of the receptor protein of the present invention (preferably neuronal cell-specific receptor protein, more preferably activin receptor protein) is preferably a physiologically acceptable acid adduct salt. Such salts include salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid) and salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid).

The receptor protein of the present invention or a salt thereof can be produced from warm-blooded animal tissue or cells by a commonly known method of protein purification, or by culturing the transformant described below, which contains DNA encoding an activin receptor protein. It can also be produced in accordance with the method of peptide synthesis described below.

The peptide of the present invention containing the amino acid sequence shown by SEQ ID NO:7 or a salt thereof can be produced by a commonly known method of peptide synthesis, or by cleaving the activin receptor protein of the present invention using an appropriate peptidase. As the peptide synthesis both solid-phase synthesis and liquid-phase synthesis are applicable. In other words, the desired peptide can be produced by condensing together a partial peptide or amino acid capable of constituting the protein of the present invention and the remaining portion, and, when the product has a protecting group, eliminating the protecting group. Useful methods of condensation or protecting group elimination are described in the following references ① through ⑤.

① M. Bodanszky and M.A. Ondetti, Peptide Synthesis, Interscience Publishers, New York (1966)

② Schroeder and Luebke, The Peptide, Academic Press, New York (1965)

③ Nobuo Izumiya et al., Peptide Gosei no Kiso to Jikken, Maruzen (1975)

④ Haruaki Yajima and Shunpei Sakakibara, Seikagaku Jikken Koza 1, Tanpakushitsu no Kagaku, 205 (1977)

⑤ Haruaki Yajima ed., Zoku Iyakuhiin no Kaihatsu, Vol. 14, Peptide Gosei, Hirokawa Shoten

The reaction may be followed by a combination of ordinary purification methods such as solvent extraction, distillation, column chromatography, liquid chromatography and recrystallization to purify and isolate the protein of the present invention. When the protein is obtained in free form, it can be converted to an appropriate salt by a known method; when the protein is obtained in salt form, it can be converted to free form by a known method.

Any DNA encoding the receptor protein of the present invention may be used, as long as it contains a base sequence encoding an activin receptor protein and containing both the amino acid sequence of the present invention of SEQ ID NO:7 and substantially the same amino acid sequence as the amino acid sequence of the amino acid sequence of SEQ ID NO:5. It may be one wherein the 5'-terminal ATG has been converted to H. It may also be genomic DNA of warm-blooded animals (e.g., humans), genomic DNA library of warm-blooded animals (e.g., humans), tissue/cell-derived cDNA of warm-blooded animals (e.g., humans), tissue/cell-derived cDNA library of warm-blooded animals (e.g., humans), or synthetic or semi-synthetic DNA. The vector used for the library may be bacteriophage, plasmid, cosmid, phagemid, or the like. Also, direct amplification by the RT-PCR method is also possible using a preparation of mRNA fraction prepared from tissue or cells. Alternatively, the desired DNA can be produced by chemically synthesizing individual partial base sequences and condensing them together.

More specifically, DNA having the base sequence shown by SEQ ID NO:4, or the like, is used as the DNA encoding the activin receptor protein from P19, a mouse embryonal carcinoma cell line, which has the amino acid sequence of SEQ ID NO:5.

Cloning of DNA encoding the entire portion of the receptor protein of the present invention can be achieved by amplifying the subject DNA by the PCR method using a synthetic DNA primer having a partial base sequence of the receptor protein, or selecting the desired DNA by hybridization of DNA incorporated in an appropriate vector with a DNA fragment having a partial or entire region of the receptor protein or with a DNA fragment labeled with synthetic DNA. This hybridization is carried out by, for example, the method described in Molecular Cloning, 2nd edition, J. Sambrook et al., Cold Spring Harbor Laboratory Press, 1989. When a commercially available library is used, hybridization is carried out as directed in the instruction manual attached to the kit. The cloned DNA encoding the receptor protein can be used as such, or after restriction enzyme digestion or linker addition as appropriate, depending on the purpose of use. Said DNA may have the translation initiation codon ATG on the 5'-terminal side thereof and the translation termination codon TAA, TGA or TAG on the 3'-terminal side thereof. These translation initiation codon and translation termination codons may also be added using an appropriate synthetic DNA adaptor.

An expression vector for the activin receptor protein can, for example, be produced by (a) excising the desired DNA fragment from DNA encoding the activin receptor protein of the present invention, and (b) ligating said DNA fragment downstream a promoter in an appropriate expression vector. Useful vectors include plasmids derived from *Escherichia coli* (e.g., pBR322, pBR325, pUC12, pUC13), plasmids derived from *Bacillus subtilis* (e.g., pUB110, pTP5, pC194), yeast-derived plasmids (e.g., pSH19, pSH15), bacteriophages such as  $\lambda$  phage, and animal viruses such as retrovirus, vaccinia virus and baculovirus.

Any promoter can be used for the present invention, as long as it is appropriate for the host used to express the desired gene.

Preferred promoters include the trp promoter, lac promoter, recA promoter,  $\lambda$ PL promoter and lpp promoter, when the transformation host is a bacterium of the genus *Escherichia*; the SPO1 promoter, SPO2 promoter and penP promoter when the host is a bacterium of the genus *Bacillus*; and the pH05 promoter, PGK promoter, GAP promoter and ADH promoter when the host is a yeast.

When the host is an animal cell, the SV40-derived promoter, retrovirus promoter, metallothionein promoter, heat shock promoter, cytomegalovirus promoter, SR $\alpha$  promoter etc. can be advantageously used. Expression is also effected by the use of an enhancer.

Also, a signal sequence corresponding to the host may be added to the N-terminal side of the receptor protein as necessary. Useful signal sequences include alkaline phosphatase signal sequence and OmpA signal sequence when the host is a bacterium of the genus *Escherichia*;  $\alpha$ -amylase signal sequence and subtilin signal sequence when the host is a bacterium of the genus *Bacillus*; the mating factor $\alpha$  signal sequence and invertase signal sequence when the host is a yeast; and the insulin signal sequence,  $\alpha$ -interferon signal sequence and antibody molecule signal sequence when the host is an animal cell.

The thus-constructed vector containing DNA encoding the receptor protein is used to produce a transformant. Examples of the host include bacteria of the genus *Escherichia*, bacteria of the genus *Bacillus*, yeasts, insects and animal cells.

Examples of the bacteria of the genus *Escherichia* include *Escherichia coli* K12 - DH1 [Proceedings of the National Academy of Sciences of the USA, Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)], HB101 [Journal of Molecular Biology, Vol. 41, 459 (1969)] and C600 [Genetics, Vol. 39, 440 (1954)].

Examples of the bacteria of the genus *Bacillus* include *Bacillus subtilis* MI114 [Gene, Vol. 24, 255 (1983)] and 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)].

Examples of the yeasts include *Saccharomyces cerevisiae* AH22, AH22R<sup>+</sup>, NA87-11A, DKD-5D and 20B-12.

Examples of the insects include silkworm larvae [Maeda et al., Nature, Vol. 315, 592 (1985)].

Examples of the animal cells include simian COS-7 cells, Vero cells, Chinese hamster CHO cells, DHFR gene deficient Chinese hamster CHO cells (dhfr<sup>-</sup> CHO cells), mouse L cells, mouse myeloma cells and human FL cells. The bacteria of the genus *Escherichia* can be transformed in accordance with the method described in the Proceedings of the National Academy of Sciences of the USA, Vol. 69, 2110 (1972) and Gene, Vol. 17, 107 (1982), for instance.

Bacteria of the genus *Bacillus* can be transformed in accordance with the method described in Molecular and General Genetics, Vol. 168, 111 (1979), for instance.

Yeasts can be transformed in accordance with the method described in the Proceedings of the National Academy of Sciences of the USA, Vol. 75, 1929 (1978), for instance.

Insect cells can be transformed in accordance with the method described in Bio/Technology, 6, 47-55 (1988), for instance.

Animal cells can be transformed in accordance with the method described in Virology, Vol. 52, 456 (1973), for instance.

A transformant resulting from transformation with an expression vector containing DNA encoding the activin receptor protein is thus obtained.

For cultivating a transformant whose host is a bacterium of the genus *Escherichia* or *Bacillus*, it is appropriate to use a liquid medium supplemented with carbon sources, nitrogen sources, minerals and other substances necessary for the growth of the transformant. Example carbon sources include glucose, dextrin, soluble starch and sucrose. Example nitrogen sources include organic or inorganic substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extract, soybean cake and potato extract. Example minerals include calcium chloride, sodium dihydrogen phosphate and magnesium chloride. Yeast extract, vitamins, growth promoters and other additives may be added. The pH of the medium is preferably about 5 to 8.

Examples of media preferably used to cultivate a bacterium of the genus *Escherichia* include the M9 medium containing glucose and casamino acid [Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold spring Harbor Laboratory, New York (1972)]. To increase promoter efficiency as necessary, a chemical agent such as 3 $\beta$ -indolyl acrylic acid may be added.

When the host is a bacterium of the genus *Escherichia*, cultivation is normally carried out at about 15 to 43°C for about 3 to 24 hours, with aeration and/or stirring as necessary.

When the host is a bacterium of the genus *Bacillus*, cultivation is normally carried out at about 30 to 40°C for about 6 to 24 hours, with aeration and/or stirring as necessary.

Examples of media for cultivating a transformant whose host is a yeast include Burkholder's minimal medium [Bostian, K.L. et al., Proceedings of the National Academy of Sciences of the USA, Vol. 77, 4505 (1980)] and SD medium containing 0.5% casamino acid [Bitter, G.A. et al., Proceedings of the National Academy of Sciences of the USA, Vol. 81, 5330 (1984)]. It is preferable to adjust the medium to a pH of about 5 to 8. Cultivation is normally carried out at about 20 to 35°C for about 24 to 72 hours, with aeration and/or stirring as necessary.

Examples of media for cultivating a transformant whose host is an insect cell include Grace's insect medium [Grace, T.C.C., Nature, 195, 788 (1962)] supplemented with additives such as 10% inactivated bovine serum as appropriate. It is preferable to adjust the medium to a pH of about 6.2 to 6.4. Cultivation is normally carried out at about 27°C for about 3 to 5 days, with aeration and/or stirring as necessary.

Examples of media for cultivating a transformant whose host is an animal cell include MEM medium containing about 5 to 20% fetal bovine serum [Science, Vol. 122, 501 (1952)], DMEM medium [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, Vol. 199, 519 (1967)] and 199 medium [Proceeding of the Society for the Biological Medicine, Vol. 73, 1 (1950)]. The pH is preferably about 6 to 8. Cultivation is normally carried out at about 30 to 40°C for about 15 to 60 hours, with aeration and/or stirring as necessary.

The receptor protein can be separated and purified from the above-described culture by, for example, the method described below.

For extracting the receptor protein from cultured cells, known methods can be used as appropriate, including the method in which cells are collected by a known method after cultivation, then suspended in an appropriate buffer, followed by cell disruption by ultrasonication, lysozyme treatment and/or freeze-thawing, after which a crude extract of receptor protein is obtained by centrifugation or filtration. The buffer may contain a protein denaturant such as urea or guanidine hydrochloride and/or a surfactant such as Triton X-100 (trade name).

When the receptor protein is secreted in the culture, the cells and supernatant are separated by a commonly known method and the latter is collected, after completion of cultivation. The receptor protein contained in the thus-obtained culture supernatant or extract can be purified by appropriate combinations of commonly known methods of separation and purification. Such known methods of separation and purification include those based on solubility differences, such as salting-out and solvent precipitation; those based mainly on molecular weight differences, such as dialysis, ultrafil-



tration, gel filtration and SDS-polyacrylamide gel electrophoresis; those based on charge differences, such as ion exchange chromatography; those based on specific affinity, such as affinity chromatography; those based on hydrophobicity differences, such as reverse-phase high performance liquid chromatography; and those based on isoelectric point differences, such as isoelectric focusing.

5 When the receptor protein is obtained in free form, it can be converted to an appropriate salt by a commonly known method or modification thereof; when the protein is obtained in salt form, it can be converted to free form by a commonly known method or modification thereof.

The receptor protein produced by a recombinant may be reacted with an appropriate protein-modifying enzyme before or after purification to modify the protein as desired or remove some polypeptides. Example protein-modifying  
10 enzymes include trypsin, chymotrypsin, arginine endopeptidase, protein kinase and glycosidase.

The activity of the resulting activin receptor protein can be determined by an experiment of binding to labeled ligand, an enzyme immunoassay using a specific antibody, etc.

The DNA encoding the receptor protein of the present invention and the receptor protein of the present invention can be used as reagent for various purposes, including ① determination of ligand against the receptor protein of the  
15 present invention, ② obtainment of antibodies and antisera, ③ construction of recombinant receptor protein expression systems, ④ development of receptor binding assay systems and screening for pharmaceutical candidate compounds using such expression systems, ⑤ drug designing based on comparison with structurally similar ligand receptors, and ⑥ reagent for preparation of probes and PCR primers for gene diagnosis, and ⑦ as a drug for gene therapy.

20 A receptor binding assay system using an expression system for the recombinant receptor protein of the present invention, in particular, makes it possible to screen for a receptor agonist or antagonist specific to warm-blooded animals, such as humans, which agonist or antagonist can be used as a prophylactic/therapeutic agent for various diseases.

Uses for the receptor protein of the present invention, salt thereof, DNA encoding said receptor protein, and anti-  
25 bodies against receptor proteins are hereinafter described specifically.

#### (1) Determination method for ligand against the receptor protein of the present invention

The receptor protein of the present invention or a salt thereof is useful as a reagent for searching or determining a  
30 ligand against the receptor protein of the present invention.

Accordingly, the present invention provides a determination method for a ligand against the receptor protein of the present invention characterized in that the receptor protein of the present invention or a salt thereof is brought into contact with a test compound.

Useful test compounds include known ligands (e.g., activin, inhibin, TGF $\beta$ , OP-1 gene product, Vg-1 gene product),  
35 and tissue extracts and cell culture supernatants of warm-blooded animals (e.g., mice, rats, pigs, bovines, sheep, monkeys, humans). For example, such a tissue extract, cell culture supernatant, or the like, may be added to the receptor protein of the present invention, followed by fractionation with monitoring of a cell-stimulating activity etc., to finally yield a single ligand.

Specifically, the ligand determination method of the present invention is carried out by using the receptor protein of  
40 the present invention or a salt thereof, or by constructing a recombinant receptor protein expression system and using a receptor binding assay system using said expression system, to determine a compound that binds to the receptor protein (e.g., peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products) to exhibit a cell-stimulating activity (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation etc.) or a salt thereof.

45 The ligand determination method of the present invention is characterized by determining the amount of test compound bound, cell-stimulating activity, or the like, to the receptor protein of the present invention or a salt thereof when said receptor protein or a salt thereof is brought into contact with the test compound.

More specifically, the present invention provides:

50 ① a method of determining a ligand against the receptor protein wherein the amount of labeled test compound bound to the receptor protein of the present invention or a salt thereof is determined when the labeled test compound is brought into contact with said protein or a salt thereof,

② a method of determining a ligand against the receptor protein wherein the amount of labeled test compound  
55 bound to cells containing the receptor protein of the present invention or the membrane fraction of said cells is determined when the labeled test compound is brought into contact with said cells or the membrane fraction thereof,

③ a method of determining a ligand against the receptor protein wherein the amount of labeled test compound bound to the receptor protein of the present invention is determined when the labeled test compound is brought into contact with said receptor protein as expressed on the cell membrane of a transformant containing DNA encoding

the receptor protein of the present invention by culturing said transformant,

④ a method of determining a ligand against the receptor protein wherein a cell-stimulating activity via receptor protein (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) is determined when a test compound is brought into contact with cells containing the receptor protein of the present invention, and

⑤ a method of determining a ligand against the receptor protein wherein a cell-stimulating activity via receptor protein (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) is determined when a test compound is brought into contact with the receptor protein as expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by culturing said transformant.

The ligand determination method of the present invention is hereinafter described specifically.

First, although any subject can be used for the ligand determination method, as long as it contains the receptor protein of the present invention, i.e., a protein containing the amino acid sequence shown by SEQ ID NO:7, receptor proteins expressed in large amounts using animal cells are suitable for screening.

An receptor protein can be produced by the above-described method, specifically by expressing DNA encoding said protein in mammalian or insect cells. DNA fragments encoding the desired portion include, but are not limited to, cDNA. For example, gene fragments and synthetic DNA may also be used. For introducing a DNA fragment encoding an receptor protein into host animal cells and efficiently expressing it, it is preferable to insert said DNA fragment downstream from the polyhedrin promoter of nuclear polyhedrosis virus (NPV) which is a baculovirus having insect hosts, SV-40-derived promoter, retrovirus promoter, metallothionein promoter, human heat shock promoter, cytomegalovirus promoter, SR $\alpha$  promoter or the like. The amount and quality of receptor expressed can be determined by commonly known methods. For example, this determination can be achieved by the method described in the literature [Nambi, P. et al., The Journal of Biological Chemistry, Vol. 267, pp. 19555-19559 (1992)].

Accordingly, the subject containing a receptor protein or a partial peptide thereof for the ligand determination method of the present invention may be the receptor protein purified by a commonly known method, a partial peptide thereof, cells containing said protein, or the membrane fraction of cells containing said protein.

When cells containing the receptor protein are used for the ligand determination method of the present invention, said cells may be immobilized using glutaraldehyde, formalin etc. This immobilization can be achieved by a commonly known method.

Cells containing the receptor protein are host cells that have expressed the receptor protein, which host cells include *Escherichia coli* cells, *Bacillus subtilis* cells, yeast cells, insect cells and animal cells.

The membrane fraction is a fraction rich in cell membrane obtained by cell disruption and subsequent fractionation by a commonly known method. Useful cell disruption methods include cell squashing using a Potter-Elvehjem homogenizer, disruption using a Waring blender or Polytron (produced by Kinematica), ultrasonic disruption, and disruption by cell spraying via a thin nozzle under increased pressure using a French press or the like. Cell membrane fractionation is achieved mainly by centrifugal fractionation methods such as fractional centrifugation and density gradient centrifugation. For example, a cell disruption liquid is centrifuged at low rate (500 to 3,000 rpm) for a short period of time (normally about 1 to 10 minutes), the resulting supernatant is then centrifuged at a higher rate (15,000 to 30,000 rpm) normally for 30 minutes to 2 hours, to yield a precipitate as the membrane fraction. The membrane fraction thus obtained is rich in the activin receptor protein expressed and membrane components such as cell-derived phospholipids and membrane proteins.

The amount of receptor protein contained in cells containing the receptor protein or membrane fraction thereof is preferably  $10^2$  to  $10^8$  molecules per cell, more preferably  $10^5$  to  $10^7$  molecules. As the amount of expression increases, the ligand binding activity per unit of membrane fraction (specific activity) increases, enabling the construction of highly sensitive screening systems and assay of large amounts of samples of the same lot.

To perform methods ① through ③ above for determination of a ligand that binds to the receptor protein, an appropriate receptor fraction and a labeled test compound are necessary. The receptor fraction is preferably a natural receptor fraction or a recombinant receptor fraction possessing equivalent activity. Here, the term equivalent activity is intended to include ligand binding activity.

Useful labeled test compounds include activin, inhibin, TGF $\alpha$ , OP-1 gene product and Vg-1 gene product all labeled with [ $^3\text{H}$ ], [ $^{125}\text{I}$ ], [ $^{14}\text{C}$ ], [ $^{35}\text{S}$ ] or the like.

Specifically, to determine a ligand that binds to the receptor protein, a standard receptor preparation is first prepared by suspending cells containing the receptor protein or the membrane fraction thereof in a buffer appropriate for the determination. Any buffer can be used, as long as it does not interfere with ligand-receptor binding, such buffers including phosphate buffers or Tris-HCl buffers of pH about 4-10 (preferably pH about 6-8). For the purpose of reducing non-specific binding, surfactants such as CHAPS, Tween-80 (trade name) (produced by Kao-Atlas), digitonin and deoxycholate, and various proteins such as bovine serum albumin and gelatin, may be added to the buffer. Also, for the purpose of suppressing receptor and ligand decomposition by protease, protease inhibitors such as PMSF (phenylmethanesulfonyl fluoride), leupeptin, E-64 (produced by Peptide Institute, Inc.) and pepstatin may be added. To 0.01-10 ml of said receptor solution, a test compound labeled with a given amount (5,000 to 500,000 cpm) of [ $^3\text{H}$ ], [ $^{125}\text{I}$ ],

[<sup>14</sup>C] or the like is added. To determine the amount of non-specific binding (NSB), a reaction tube containing an unlabeled test compound in excess is also provided. Reaction is carried out at 0 to 50°C, preferably 4 to 37°C for 20 minutes to 24 hours, preferably 30 minutes to 3 hours. After completion of the reaction, the reaction mixture is filtered through glass fiber filter paper etc. and washed with an appropriate amount of the same buffer, after which the residual radioactivity in the glass fiber filter paper is measured using a liquid scintillation counter or γ-counter. A test compound exceeding 0 cpm in count (B - NSB) obtained by subtracting nonspecific binding (NSB) from total binding (B) may be selected as a ligand against the activin receptor protein of the present invention.

To perform method ④ or ⑤ above for determination of a ligand against the receptor protein, a cell-stimulating activity via receptor protein (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) may be determined using a known method or a commercially available assay kit. Specifically, cells containing the receptor protein are first cultured on multiwell plates etc. Prior to ligand determination, the medium is replaced with fresh medium or an appropriate non-cytotoxic buffer, followed by incubation in the presence of a test compound etc. for a given period of time, after which cells are extracted or the supernatant is recovered, and the resulting product is quantified as appropriate. When it is difficult to detect the production of the cell-stimulating activity index substance (e.g., plasminogen activator inhibitor 1, fibronectin) due to a decomposing enzyme contained in the cells, an inhibitor against said decomposing enzyme may be added before assay.

The kit of the present invention for determination of a ligand that binds to the receptor protein of the present invention comprises the receptor protein of the present invention or a salt thereof, cells containing the receptor protein of the present invention, or the membrane fraction thereof.

Some examples of the ligand determination kit of the present invention are given below.

#### 1. Ligand determination reagents

##### ① Assay buffer and washing buffer

Hanks' balanced salt solution (produced by GIBCO) supplemented with 0.05% bovine serum albumin (produced by Sigma Corporation).

After being sterilized by filtration through a filter of 0.45 μm in pore size, this buffer may be stored at 4°C, or may be prepared freshly at each use.

##### ② Standard activin receptor protein preparation

CHO cells containing a receptor protein expressed therein are subcultured at  $5 \times 10^5$  cells/well on 12-well plates at 37°C in the presence of 5% CO<sub>2</sub> and 95% air for 2 days.

##### ③ Labeled test compound

Compound labeled with commercially available [<sup>3</sup>H], [<sup>125</sup>I], [<sup>14</sup>C], [<sup>35</sup>S] or the like, or compound labeled by an appropriate method.

The labeled test compound in aqueous solution is stored at 4°C or -20°C, and diluted to 1 μM with the assay buffer before use. When the test compound is practically insoluble in water, it must be dissolved in dimethylformamide, DMSO, methanol, or the like.

##### ④ Standard liquid solution

The labeled compound obtained in term ③ above is diluted to 1 mM with PBS containing 0.1% bovine serum albumin (produced by Sigma Corporation).

#### 2. Method of assay

① After CHO cells containing a receptor protein expressed therein, cultured on 12-well tissue culture plates, are twice washed with 1 ml of the assay buffer, 490 μl of the assay buffer is added to each well.

② Five microliter of the labeled test compound is added, followed by reaction at room temperature for 1 hour. To determine the amount of non-specific binding, 5 μl of the unlabeled test compound is added before reaction.

③ After the reaction mixture is removed, the plate is washed 3 times with 1 ml of the washing buffer. The labeled test compound bound to cells is dissolved in 0.2 N NaOH-1% SDS and mixed with 4 ml of liquid scintillator A (produced by Wako Pure Chemical Industries).

④ Radioactivity is determined using a liquid scintillation counter (produced by Beckman).

#### (2) Prophylactic/therapeutic agent for deficiency of the receptor protein of the present invention

If a ligand against the receptor protein of the present invention is demonstrated by method (1) above, DNA encoding the receptor protein of the present invention could be used as a prophylactic/therapeutic agent against activin receptor protein deficiency according to the action of said ligand.

For example, for a patient in whom the physiological action of ligand is not expected due to decreased content of the receptor protein of the present invention in the body, the receptor protein content in the patient can be increased to

ensure satisfactory ligand action by, for example, (a) administering DNA encoding the receptor protein of the present invention to the patient and expressing the receptor protein in the body, or (b) inserting DNA encoding the receptor protein of the present invention to brain cells etc., and expressing it there, then transplanting the brain cells etc. to the patient. DNA encoding the receptor protein of the present invention can therefore be used as a safe prophylactic/therapeutic agent for activin receptor protein deficiency of low toxicity.

When the DNA of the present invention is used as a prophylactic/therapeutic agent as described above, the DNA may be used as such or after being inserted to an appropriate vector such as the retrovirus vector, adenovirus vector or adenovirus-associated virus vector, followed by a conventional method of drug administration. For example, the DNA can be used orally in the form of tablets, capsules, elixirs, microcapsules etc., all of which may be sugar coated as necessary, or non-orally in the form of injectable preparations such as aseptic solutions and suspensions in water or other pharmaceutically acceptable liquids. These preparations can be produced by mixing the DNA of the present invention with physiologically acceptable carriers, flavoring agents, excipients, vehicles, antiseptics, stabilizers, binders etc. in unit dosage forms for generally accepted manners of pharmaceutical making. Active ingredient contents in these preparations are set so that an appropriate dose within the specified range is obtained.

Additives which can be mixed in tablets, capsules etc. include binders such as gelatin, corn starch, tragacanth and gum arabic, excipients such as crystalline cellulose, swelling agents such as corn starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetening agents such as sucrose, lactose and saccharin, and flavoring agents such as peppermint and cherry. When the unit dosage form is the capsule, the above-mentioned materials may further incorporate liquid carriers such as oils and fats. Sterile compositions for injection can be formulated by ordinary methods of pharmaceutical making such as by dissolving or suspending active ingredients, naturally occurring vegetable oils such as sesame oil and coconut oil, etc. in vehicles such as water for injection. Aqueous liquids for injection include physiological saline and isotonic solutions containing glucose and other auxiliary agents (e.g., D-sorbitol, D-mannitol, sodium chloride), and may be used in combination with appropriate dissolution aids such as alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., polysorbate 80 (trade name), HCO-50) etc. Oily liquids include sesame oil and soybean oil, and may be used in combination with dissolution aids such as benzyl benzoate and benzyl alcohol.

The aqueous liquid may also be formulated with buffers (e.g., phosphate buffer, sodium acetate buffer), soothing agents (e.g., benzalkonium chloride, procaine hydrochloride), stabilizers (e.g., human serum albumin, polyethylene glycol), preservatives (e.g., benzyl alcohol, phenol), antioxidants etc. The thus-prepared injectable liquid is normally filled in an appropriate ampule. Because the thus-obtained preparation is safe and of low toxicity, it can be administered to warm-blooded mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, humans), for instance. The dose of said DNA is normally about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg per day for an adult (weighing 60 kg) in oral administration, depending on symptoms etc. In non-oral administration, it is advantageous to administer the DNA in the form of injectable preparation at a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg per administration for an adult (weighing 60 kg), depending on subject of administration, target organ, symptoms, method of administration etc. For other animal species, corresponding doses as converted per 60 kg weight can be administered.

### (3) Quantitative determination method for ligand against the receptor protein of the present invention

The receptor protein of the present invention or a salt thereof enables highly sensitive quantitative determination of ligand concentration in the body because it is capable of binding to ligand.

The quantitative determination method of the present invention can be used in combination with competitive assay, for example. In other words, the ligand concentration in the sample can be determined by bringing it into contact with the receptor protein of the present invention or a salt thereof. Specifically, it can be performed by the methods described in references ① and ② below or modifications thereof.

① Hiroshi Irie ed., Radioimmunoassay, Kodansha, 1974.

② Hiroshi Irie ed., Zoku Radioimmunoassay, Kodansha, 1979.

### (4) Screening method for compounds that inhibit or promote the binding of the receptor protein of the present invention and ligand

By using the receptor protein of the present invention or a salt thereof, or by constructing a recombinant receptor protein expression system and using a receptor binding assay system using said expression system, it is possible to screen for a compound that inhibits or promotes the binding of a ligand and the receptor protein (e.g., peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products) or for a salt thereof. Such compounds include compounds that exhibit a cell-stimulating activity via receptor (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation), and compounds lacking said cell-stimulating activity (what is called receptor

antagonists of the present invention).

Accordingly, the present invention provides a method of screening for a compound that inhibits or promotes the binding of a ligand and the receptor protein of the present invention or a salt thereof, wherein comparison is made between case (i) in which the ligand is brought into contact with the receptor protein of the present invention or a salt thereof, and case (ii) in which both the ligand and a test compound are brought into contact with the receptor protein of the present invention or a salt thereof.

The screening method of the present invention is characterized by comparison of, for example, the amount of ligand bound to the receptor protein of the present invention or a salt thereof, or cell-stimulating activity, between case (i) in which the ligand is brought into contact with the receptor protein of the present invention or a salt thereof, and case (ii) in which both the ligand and a test compound are brought into contact with the receptor protein of the present invention or a salt thereof.

More specifically, the present invention provides:

① a method of screening for a compound that inhibits or promotes the binding of a ligand and the receptor protein of the present invention or a salt thereof, or for a salt thereof, wherein the amount of labeled ligand bound to said protein or a salt thereof, is compared between a case in which the labeled ligand is brought into contact with the receptor protein of the present invention or a salt thereof, and another case in which both the labeled ligand and a test compound are brought into contact with the receptor protein of the present invention or a salt thereof,

② a method of screening for a compound that inhibits or promotes the binding of a ligand and the receptor protein of the present invention, or for a salt thereof, wherein the amount of labeled ligand bound to cells containing the receptor protein of the present invention is compared between a case in which the labeled ligand is brought into contact with said cells or the membrane fraction thereof and another case in which both the labeled ligand and a test compound are brought into contact with said cells or the membrane fraction thereof,

③ a method of screening for a compound that inhibits or promotes the binding of a ligand and the receptor protein of the present invention, or for a salt thereof, wherein the amount of labeled ligand bound to said receptor protein is compared between a case in which the labeled ligand is brought into contact with said receptor protein as expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by culturing said transformant, and another case in which both the labeled ligand and a test compound are brought into contact with said receptor protein as expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by culturing said transformant,

④ a method of screening for a compound that inhibits or promotes the binding of a ligand and the activin receptor protein of the present invention, or for a salt thereof, wherein a cell-stimulating activity via activin receptor protein (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) is compared between a case in which a compound that activates the receptor protein of the present invention (e.g., ligand against the receptor protein of the present invention) is brought into contact with cells containing the receptor protein of the present invention, and another case in which both the compound that activates the receptor of the present invention and a test compound are brought into contact with cells containing the receptor protein of the present invention, and

⑤ a method of screening for a compound that inhibits or promotes the binding of a ligand and the activin receptor protein of the present invention, or for a salt thereof, wherein a cell-stimulating activity via receptor (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) is compared between a case in which a compound that activates the receptor of the present invention (e.g., ligand against the activin receptor protein of the present invention) is brought into contact with the receptor protein as expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by culturing said transformant, and another case in which both the compound that activates the receptor protein of the present invention and a test compound are brought into contact with the receptor protein as expressed on the cell membrane of a transformant containing DNA encoding the receptor protein of the present invention by culturing said transformant.

The screening method of the present invention is hereinafter described specifically.

First, although any subject can be used for the screening method of the present invention, as long as it contains the receptor protein of the present invention or a salt thereof, preference is given to membrane fractions of organs of warm-blooded animals. Because human organs are very difficult to obtain, however, receptor proteins expressed in large amounts using recombinants are suitable for screening.

A receptor protein can be produced by the above-described method, specifically by expressing DNA encoding said protein in mammalian or insect cells. DNA fragments encoding the desired portion include, but are not limited to, complementary DNA. For example, gene fragments and synthetic DNA may also be used. For introducing a DNA fragment encoding a receptor protein into host animal cells and efficiently expressing it, it is preferable to insert said DNA fragment downstream from the polyhedrin promoter of nuclear polyhedrosis virus (NPV) which is a baculovirus having insect hosts, SV-40-derived promoter, retrovirus promoter, metallothionein promoter, human heat shock promoter,

cytomegalovirus promoter, SR $\alpha$  promoter or the like. The amount and quality of receptor expressed can be determined by commonly known methods. For example, this determination can be achieved by the method described in the literature [Nambi, P. et al., The Journal of Biological Chemistry, Vol. 267, pp. 19555-19559 (1992)].

Accordingly, the subject containing a receptor protein or a salt thereof for the screening method of the present invention may be a receptor protein purified by a commonly known method, cells containing said protein, or the membrane fraction of cells containing said protein.

When cells containing a receptor protein are used for the screening method of the present invention, said cells may be immobilized using glutaraldehyde, formalin etc. This immobilization can be achieved by a commonly known method.

Cells containing a receptor protein are host cells that have expressed the receptor protein, which host cells include *Escherichia coli* cells, *Bacillus subtilis* cells, yeast cells, insect cells and animal cells.

The membrane fraction is a fraction rich in cell membrane obtained by cell disruption and subsequent fractionation by a commonly known method. Useful cell disruption methods include cell squashing using a Potter-Elvehjem homogenizer, disruption using a Waring blender or Polytron (produced by Kinematica), ultrasonic disruption, and disruption by cell spraying via a thin nozzle under increased pressure using a French press or the like. Cell membrane fractionation is achieved mainly by centrifugal fractionation methods such as fractional centrifugation and density gradient centrifugation. For example, a cell disruption liquid is centrifuged at low rate (500 to 3,000 rpm) for a short period of time (normally about 1 to 10 minutes), the resulting supernatant is then centrifuged at higher rate (15,000 to 30,000 rpm) normally for 30 minutes to 2 hours, to yield a precipitate as the membrane fraction. The membrane fraction thus obtained is rich in the receptor protein expressed and membrane components such as cell-derived phospholipids and membrane proteins.

The amount of receptor protein contained in cells containing the receptor protein or membrane fraction thereof is preferably  $10^2$  to  $10^8$  molecules per cell, more preferably  $10^5$  to  $10^7$  molecules. As the amount of expression increases, the ligand binding activity per unit of membrane fraction (specific activity) increases, enabling the construction of highly sensitive screening systems and assay of large amounts of samples of the same lot.

To perform screening methods ① through ③ above for a compound that inhibits the binding of a ligand and the receptor protein of the present invention, an appropriate receptor fraction and a labeled ligand are necessary. The receptor fraction is preferably a natural receptor fraction or a recombinant receptor fraction possessing equivalent activity. Here, the term equivalent activity is intended to include ligand binding activity.

Useful labeled ligands include labeled ligands and labeled ligand analog compounds, such as those labeled with [ $^3\text{H}$ ], [ $^{125}\text{I}$ ], [ $^{14}\text{C}$ ], [ $^{35}\text{S}$ ] or the like.

Specifically, to screen for a compound that inhibits the binding of a ligand and a receptor protein, a standard receptor preparation is first prepared by suspending cells containing the receptor protein or the membrane fraction thereof in a buffer appropriate for the screening. Any buffer can be used, as long as it does not interfere with ligand-receptor binding, such buffers including phosphate buffers and Tris-HCl buffers of pH about 4-10 (preferably pH about 6-8). For the purpose of reducing non-specific binding, surfactants such as CHAPS, Tween80 (trade name) (produced by Kao-Atlas), digitonin and deoxycholate may be added to the buffer. Also, for the purpose of suppressing receptor and ligand decomposition by protease, protease inhibitors such as PMSF, leupeptin, E-64 (produced by Peptide Institute, Inc.) and pepstatin may be added. To 0.01-10 ml of said receptor solution, a ligand labeled with a given amount (5,000 to 500,000 cpm) of marker is added. Also added is a test compound at  $10^{-4}$  M to  $10^{-10}$  M. To determine the amount of non-specific binding (NSB), a reaction tube containing an unlabeled ligand in excess is also provided. Reaction is carried out at 0 to 50°C, preferably 4 to 37°C for 20 minutes to 24 hours, preferably 30 minutes to 3 hours. After completion of the reaction, the reaction mixture is filtered through glass fiber filter paper etc. and washed with an appropriate amount of the same buffer, after which the residual radioactivity in the glass fiber filter paper is measured using a liquid scintillation counter or  $\gamma$ -counter. A test compound not higher than 50%, for instance, in amount of specific binding (B - NSB), relative to the count obtained by subtracting nonspecific binding (NSB) from the count in the absence of antagonists (B0) (B0 - NSB), may be selected as a candidate substance having potential for antagonism; a test compound not lower than 150%, for instance, in amount of specific binding (B - NSB), may be selected as a candidate substance having potential for promotion of binding.

To perform screening method ④ or ⑤ above for a compound that inhibits or promotes the binding of a ligand and the receptor protein of the present invention, a cell-stimulating activity via receptor protein (e.g., growth promotion, promotion or suppression of intracellular protein phosphorylation) may be determined using a known method or a commercially available assay kit. Specifically, cells containing the receptor protein are first cultured on multiwell plates etc. Prior to screening, the medium is replaced with fresh medium or an appropriate non-cytotoxic buffer, followed by incubation in the presence of a test compound etc. for a given period of time, after which cells are extracted or the supernatant is recovered, and the resulting product is quantified as appropriate. When it is difficult to detect the production of the cell-stimulating activity index substance (e.g., plasminogen activator inhibitor 1, fibronectin) due to a decomposing enzyme contained in the cells, an inhibitor against said decomposing enzyme may be added before assay.

For screening based on cell-stimulating activity determination, cells containing an appropriate receptor protein expressed therein are necessary. Preferable cells containing the receptor protein of the present invention expressed

therein include a cell line having the receptor protein of the present invention in natural form (e.g., mouse embryonic tumor cell line P19) and a cell line that expresses a recombinant receptor protein.

Useful test compounds include peptides, proteins, non-peptide compounds, synthetic compounds, fermentation products, cell extract, plant extract and animal tissue extract, and may be new compounds or known compounds.

The screening kit for a compound that inhibits or promotes the binding of a ligand and the receptor protein of the present invention or a salt thereof comprises the receptor protein of the present invention or a salt thereof, a partial peptide thereof, or a salt thereof, cells containing the receptor protein of the present invention, or the membrane fraction thereof.

Some examples of the screening kit of the present invention are given below.

## 1. Screening reagents

### ① Assay buffer and washing buffer

Hanks' balanced salt solution (produced by GIBCO) supplemented with 0.05% bovine serum albumin (produced by Sigma Corporation)

After being sterilized by filtration through a filter of 0.45  $\mu\text{m}$  in pore size, this buffer may be stored at 4°C, or may be prepared freshly at each use.

### ② Standard receptor protein preparation

CHO cells containing a receptor protein expressed therein are subcultured at  $5 \times 10^5$  cells/well on 12-well plates at 37°C in the presence of 5%  $\text{CO}_2$  and 95% air for 2 days at 4°C or -20°C, and diluted to 1  $\mu\text{M}$  with the assay buffer before use.

### ③ Labeled ligand

Aqueous ligand solution labeled with commercially available [ $^3\text{H}$ ], [ $^{125}\text{I}$ ], [ $^{14}\text{C}$ ], [ $^{35}\text{S}$ ] or the like is stored.

### ④ Standard ligand solution

The ligand is dissolved to 1 mM in PBS containing 0.1% bovine serum albumin (produced by Sigma Corporation) and stored at -20°C.

## 2. Method of assay

① After CHO cells containing a receptor protein expressed therein, cultured on 12-well tissue culture plates, are twice washed with 1 ml of the assay buffer, 490  $\mu\text{l}$  of the assay buffer is added to each well.

② After 5  $\mu\text{l}$  of the test compound is added at  $10^{-3}$  to  $10^{-10}$  M, 5  $\mu\text{l}$  of the labeled ligand is added, followed by reaction at room temperature for 1 hour. To determine the amount of non-specific binding, 5  $\mu\text{l}$  of the unlabeled ligand at  $10^{-3}$  M, in place of the test compound, is added before reaction.

③ After the reaction mixture is removed, the plate is washed 3 times with 1 ml of the washing buffer. The labeled ligand bound to cells is dissolved in 0.2 N NaOH-1% SDS and mixed with 4 ml of liquid scintillator A (produced by Wako Pure Chemical Industries).

④ Radioactivity is determined using a liquid scintillation counter (produced by Beckman). Percent maximum binding (PMB) is calculated using equation 1 below.

Equation 1

$$\text{PMB} = [(B - \text{NSB}) / (B_0 - \text{NSB})] \times 100$$

PMB: Percent maximum binding

B : Value obtained in presence of sample

NSB: Non-specific binding

B<sub>0</sub> : Maximum binding

The compound or salt thereof obtained using the screening method or screening kit of the present invention is a compound that inhibits or promotes the binding of a ligand and the receptor of the present invention, specifically a compound possessing a cell-stimulating activity via receptor or salt thereof (what is called activin receptor agonist) or a compound not possessing such cell-stimulating activity (what is called activin receptor antagonist). Useful test compounds include peptides, proteins, non-peptide compounds, synthetic compounds and fermentation products, and may be new compounds or known compounds.

Because said receptor agonist processes the same bioactivity as that of the ligand against the receptor protein of the present invention, it is useful as a safe pharmaceutical composition of low toxicity, especially a prophylactic/therapeutic agent for nerve degeneration disease, according to said ligand activity.

Conversely, because said receptor antagonists are capable of suppressing the bioactivity of a ligand against the receptor protein of the present invention, they are useful as safe pharmaceutical compositions of low toxicity, especially

prophylactic/therapeutic agents for nerve degeneration disease, in suppressing said ligand bioactivity.

When the compound obtained using the screening method or screening kit of the present invention or a salt thereof is used as a pharmaceutical composition as described above, it can be used safely at low toxicity by conventional methods. For example, it can be used orally in the form of tablets, capsules, elixirs, microcapsules etc., all of which may be sugar coated as necessary, or non-orally in the form of injectable preparations such as aseptic solutions and suspensions in water or other pharmaceutically acceptable liquids. These preparations can be produced by mixing the compound or salt thereof with physiologically acceptable carriers, flavoring agents, excipients, vehicles, antiseptics, stabilizers, binders etc. in unit dosage forms for generally accepted manners of pharmaceutical making. Active ingredients in these preparations are set so that an appropriate dose within the specified range is obtained.

Additives which can be mixed in tablets, capsules etc. include binders such as gelatin, corn starch, tragacanth and gum arabic, excipients such as crystalline cellulose, swelling agents such as corn starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweetening agents such as sucrose, lactose and saccharin, and flavoring agents such as peppermint and cherry. When the unit dosage form is the capsule, the above-mentioned materials may further incorporate liquid carriers such as oils and fats. Sterile compositions for injection can be formulated by ordinary methods of pharmaceutical making such as by dissolving or suspending active ingredients, naturally occurring vegetable oils such as sesame oil and coconut oil, etc. in vehicles such as water for injection.

Aqueous liquids for injection include physiological saline and isotonic solutions containing glucose and other auxiliary agents (e.g., D-sorbitol, D-mannitol, sodium chloride), and may be used in combination with appropriate dissolution aids such as alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., polysorbate 80 (trade name), HCO-50) etc. Oily liquids include sesame oil and soybean oil, and may be used in combination with dissolution aids such as benzyl benzoate and benzyl alcohol. The aqueous liquid may also be formulated with buffers (e.g., phosphate buffer, sodium acetate buffer), soothing agents (e.g., benzalkonium chloride, procaine hydrochloride), stabilizers (e.g., human serum albumin, polyethylene glycol), preservatives (e.g., benzyl alcohol, phenol), antioxidants etc. The thus-prepared injectable liquid is normally filled in an appropriate ampule. Because the thus-obtained preparation is safe and of low toxicity, it can be administered to warm-blooded mammals (e.g., rats, rabbits, sheep, pigs, bovines, cats, dogs, monkeys, humans), for instance.

The dose of said compound or a salt thereof is normally about 0.1 to 100 mg, preferably about 1.0 to 50 mg, and more preferably about 1.0 to 20 mg per day for an adult (weighing 60 kg) in oral administration, depending on symptoms etc. In non-oral administration, it is advantageous to administer the compound or a salt thereof in the form of injectable preparation at a daily dose of about 0.01 to 30 mg, preferably about 0.1 to 20 mg, and more preferably about 0.1 to 10 mg per administration for an adult (weighing 60 kg), depending on subject of administration, target organ, symptoms, method of administration etc. For other animal species, corresponding doses as converted per 60 kg weight can be administered.

(5) Production of antibody or antiserum against the receptor protein of the present invention, partial peptide thereof, or salt thereof

An antibody (e.g., polyclonal antibody, monoclonal antibody) or antiserum against the receptor protein of the present invention or a salt thereof, can be produced by a commonly known method of antibody or antiserum production using the receptor protein of the present invention or a salt thereof as an antigen. For example, a monoclonal antibody can be produced by the method described below. Preparation of monoclonal antibody

#### (a) Preparation of monoclonal antibody producer cells

The receptor protein of the present invention, a partial peptide thereof, or a salt thereof (hereinafter also referred to as activin receptor) is administered to warm-blooded animals at a site permitting antibody production by its administration, as such or in combination with a carrier, diluent etc. To increase antibody productivity, Freund's complete or incomplete adjuvant may be administered. Administration is normally performed about 2 to 10 times in total once every 2 to 6 weeks.

Although useful warm-blooded animals include monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, goat and chickens, mice and rats are preferred.

For preparing monoclonal antibody producer cells, animals showing an antibody titer are selected from antigen-immunized warm-blooded animals, e.g., mice; 2 to 5 days after final immunization, spleens or lymph nodes are collected; the antibody-producing cells contained therein are fused with myeloma cells to yield a monoclonal antibody producer hybridoma. Antibody titer in the antiserum is determined by reacting a labeled receptor as described below and the antiserum, then determining the activity of marker bound to the antibody. Fusion can be achieved by a known method such as the method of Kohler and Milstein [Nature, 256, 495 (1975)]. Although useful fusogens include polyethylene glycol (PEG) and Sendai virus, PEG is preferred.

Although useful myeloma cell lines include NS-1, P3U1, SP2/0 and AP-1, P3U1 is preferred. The number ratio of



antibody-producing cells (splenocytes) and myeloma cells used is preferably about 1:1 to 20:1; cell fusion is facilitated when incubation is conducted at 20-40°C, preferably 30-37°C for 1 to 10 minutes in the presence of about 10-80% PEG (preferably PEG1000 to PEG6000).

Although various methods are applicable to screening for hybridomas that produce an anti-activin receptor antibody, including the method in which a hybridoma culture supernatant is added to a solid phase (e.g., microplate) to which a receptor antigen is previously adsorbed directly or via a carrier, after which an anti-immunoglobulin antibody (anti-mouse immunoglobulin antibody in the case of mouse cells for cell fusion) or protein A labeled with a radioactive substance, enzyme or the like is added to detect the anti receptor monoclonal antibody bound to the solid phase, and the method in which a hybridoma culture supernatant is added to a solid phase to which an anti-immunoglobulin antibody or protein A is previously adsorbed, after which a receptor labeled with a radioactive substance, enzyme or the like is added to detect the anti receptor monoclonal antibody bound to the solid phase.

Selection of anti receptor monoclonal antibodies can be achieved by a commonly known method or modification thereof, normally in an animal cell culture medium supplemented with HAT (hypoxanthine, aminopterin, thymidine). Any selection and breeding media can be used, as long as they allow the hybridoma to grow. Useful media include RPMI 1640 medium containing 1-20%, preferably 10-20% fetal calf serum, GIT medium containing 1-10% fetal calf serum (Wako Pure Chemical Industries) and serum-free medium for hybridoma culture (SFM-101, Nissui Pharmaceutical). Culturing temperature is normally 20-40°C, preferably about 37°C. Culturing time is normally 5 days to 3 weeks, preferably 1 to 2 weeks. Cultivation is normally carried out in the presence of 5% gaseous carbon dioxide. Antibody titer of the hybridoma culture supernatant can be determined in the same manner as for the anti receptor antibody titer in the antiserum.

#### (b) Purification of monoclonal antibody

An anti receptor monoclonal antibody can be separated and purified by methods of immunoglobulin separation and purification [e.g., salting-out, alcohol precipitation, isoelectric precipitation, electrophoresis, adsorption/desorption using ion exchanger (e.g., DEAE), ultracentrifugation, gel filtration, and specific purification in which the desired antibody alone is collected using an active adsorbent such as antigen-bound solid phase, protein A, protein G or the like, followed by breaking the bond], as in ordinary separation and purification of polyclonal antibodies.

Because the receptor antibody of the present invention as produced by method (a) or (b) above is capable of specifically recognizing the receptor, it can be used to quantify a receptor in the subject solution, especially by sandwich immunometry etc.

Accordingly, the present invention provides, for example:

- (i) a method of quantifying a receptor in a subject solution wherein an antibody reactive with the receptor of the present invention, the subject solution, and a labeled receptor are competitively reacted together, and the ratio of labeled receptor bound to the antibody is determined, and
- (ii) a method of quantifying a receptor in a subject solution by simultaneously or sequentially reacting the subject solution, an antibody insolubilized on a carrier and a labeled antibody, and determining the activity of the marker on the insolubilizing carrier, wherein one antibody recognizes the N- or C-terminal portion of the receptor and the other is reactive with the amino acid sequence of SEQ ID NO:7.

The monoclonal antibody of the present invention, which recognizes a receptor (hereinafter also referred to as anti receptor antibody) can be used not only to measure the receptor but also to detect the receptor by tissue staining etc. For these purposes, the antibody molecular may be used as such or as the F(ab')<sup>2</sup>, Fab' or Fab fraction thereof. When using the antibody of the present invention, any assay method can be employed, as long as the amount of antibody, antigen or antibody-antigen complex corresponding to the amount of antigen (e.g., amount of activin receptor) in the subject solution is chemically or physically detected and calculated on a standard curve drawn using a standard solution containing a known amount of antigen. Although useful assay methods include nephrometry, competitive assay, immunometry and the sandwich method, the sandwich method described below is particularly preferable in terms of sensitivity and specificity.

Markers which can be used for assay methods using a labeled substance include radioisotopes, enzymes, fluorescent substances and luminescent substances. Preferable radioisotopes include [<sup>125</sup>I], [<sup>131</sup>I], [<sup>3</sup>H] and [<sup>14</sup>C]. Such enzymes are preferably stable and of high specific activity, exemplified by  $\beta$ -galactosidase,  $\beta$ -glucosidase, alkaline phosphatase, peroxidase and malic acid dehydrogenase. Useful fluorescent substances include fluorescamine and fluorescein isothiocyanate. Useful luminescent substances include luminol, luminol derivatives, luciferin and Lucigenin. Also, biotin-avidin systems can be used to bind an antibody or antigen and a marker.

Antigen or antibody insolubilization may be achieved using physical adsorption or by a commonly used method based on chemical bonds for insolubilization and immobilization of proteins, enzymes etc. Useful carriers include insoluble polysaccharides such as agarose, dextran and cellulose, synthetic resins such as polystyrene, polyacrylamide and

silicone, and glass. In the sandwich method, a subject solution is reacted with an insolubilized anti receptor antibody (primary reaction), followed by another reaction of a labeled anti receptor antibody (secondary reaction), after which the activity of marker on the insolubilizing carrier is determined to obtain the amount of receptor in the subject solution. The primary and secondary reactions may be carried out in the reverse order, simultaneously, or at a time interval. The marker and insolubilization method used may be the same as those described above. In immunometry by the sandwich method, solid phase antibodies or marker antibodies need not be used singly; they may be used in a mixture of two or more such antibodies to improve assay sensitivity or for other purposes.

In the assay method for receptor by the sandwich method of the present invention, the anti receptor antibodies used in the primary and secondary reactions are preferably antibodies having mutually different receptor binding sites. In other words, when the antibody for the secondary reaction recognizes the C- or N-terminal portion of the receptor, the antibody for the primary reaction preferably recognizes the amino acid sequence shown by SEQ ID NO:7.

The receptor antibody of the present invention can be used for assay systems other than those based on the sandwich method, such as competitive assay, immunometry or nephrometry. In competitive assay, an antigen and labeled antigen in a subject solution are competitively reacted with an antibody, after which the unreacted labeled antigen (F) and antibody-bound labeled antigen (B) are separated (B/F separation), the amount of marker activity in either B or F is then determined to obtain the amount of antigen in the subject solution. This reaction employs either the liquid phase method using a soluble antibody and using polyethylene glycol, a secondary antibody against the above antibody etc. for B/F separation, or the solid phase method using a primary antibody immobilized on a solid phase, or using a soluble primary antibody and a secondary antibody immobilized on a solid phase.

In immunometry, an antigen and immobilized antigen on solid phase in the subject solution are competitively reacted with a given amount of labeled antibody, followed by separation of the solid and liquid phases, or the antigen in the subject solution is reacted with an excess of labeled antibody, then an immobilized antigen on solid phase is added to bind the unreacted labeled antibody to the solid phase, followed by separation of the solid and liquid phases. The amount of marker activity in either phase is then determined to obtain the amount of antigen in the subject solution.

In nephrometry, the amount of insoluble precipitate resulting from antigen-antibody reaction in gel or solution is determined. Even when the amount of antigen in the subject solution is so small that only a small amount of precipitate is obtained, laser nephrometry, based on laser scattering, etc. is preferably used.

In applying these immunological assays to the assay method of the present invention, there is no need for special conditions, procedures etc. A receptor assay system may be constructed on the basis of ordinary conditions, procedures etc. for respective methods, in consideration of ordinary technical aspects known to those skilled in the art. For details of these common technical approaches, published overviews, books etc. may serve as references [see e.g., "Radioimmunoassay," Hiroshi Irie ed., Kodansha, 1974, "Zoku Radioimmunoassay," Hiroshi Irie ed., Kodansha, 1979, "Koso Meneki Sokuteihou," Eiji Ishikawa et al. ed., Igaku-Shoin Ltd., 1978, "Koso Meneki Sokuteihou," 2nd edition, Eiji Ishikawa et al. ed., Igaku-Shoin Ltd., 1982, "Koso Meneki Sokuteihou," 3rd edition, Eiji Ishikawa et al. ed., Igaku-Shoin Ltd., 1987, "Methods in Enzymology," Vol. 70 (Immunochemical Techniques, Part A), *ibid.*, Vol. 73 (Immunochemical Techniques, Part B), *ibid.*, Vol. 74 (Immunochemical Techniques, Part C), *ibid.*, Vol. 84 (Immunochemical Techniques, Part D: Selected Immunoassays), *ibid.*, Vol. 92 (Immunochemical Techniques, Part E: Monoclonal Antibodies and General Immunoassay Methods), *ibid.*, Vol. 121 (Immunochemical Techniques, Part I: Hybridoma Technology and Monoclonal Antibodies), all published by Academic Press].

The receptors can be quantified at high sensitivity using the receptor antibody of the present invention as described above.

Abbreviations for bases, amino acids and others used in the present specification and attached drawings are based on abbreviations specified by the IUPAC-IUB Commission on Biochemical Nomenclature or abbreviations in common use in relevant fields. Some examples are given below. When an optical isomer may be present in amino acid, it is of the L-configuration, unless otherwise stated.

DNA :	Deoxyribonucleic acid
cDNA:	Complementary deoxyribonucleic acid
A :	Adenine
T :	Thymine
G :	Guanine
C :	Cytosine
RNA :	Ribonucleic acid
mRNA:	Messenger ribonucleic acid
dATP:	Deoxyadenosine triphosphate
dTTP:	Deoxythymidine triphosphate
dGTP:	Deoxyguanosine triphosphate
dCTP:	Deoxycytidine triphosphate
ATP :	Adenosine triphosphate

	EDTA:	Ethylenediaminetetraacetic acid
	SDS :	Sodium dodecyl sulfate
	EIA :	Enzyme immunoassay
	Gly :	Glycine
5	Ala :	Alanine
	Val :	Valine
	Leu :	Leucine
	Ile :	Isoleucine
	Ser :	Serine
10	Thr :	Threonine
	Cys :	Cysteine
	Met :	Methionine
	Glu :	Glutamic acid
	Asp :	Aspartic acid
15	Lys :	Lysine
	Arg :	Arginine
	His :	Histidine
	Phe :	Phenylalanine
	Tyr :	Tyrosine
20	Trp :	Tryptophan
	Pro :	Proline
	Asn :	Asparagine
	Gln :	Glutamine
	pGI :	Pyroglutamine
25	Me :	Methyl group
	Et :	Ethyl group
	Bu :	Butyl group
	Ph :	Phenyl group
	TC :	Thiazolidine-4(R)-carboxamide group

30 Sequence Identification Numbers in the sequence table in the present specification represent the following sequences:

SEQ ID NO:1

35 The base sequence of a synthetic DNA primer used to amplify cDNA encoding the receptor protein of the present invention by the RT-PCR method.

SEQ ID NO:2

40 The base sequence of a synthetic DNA primer used to amplify cDNA encoding the receptor protein of the present invention by the RT-PCR method.

SEQ ID NO:3

45 The base sequence of cDNA from mouse embryonal carcinoma cell line P19 encoding eight amino acid sequences characteristic of the receptor protein of the present invention.

SEQ ID NO:4

50 The base sequence of cDNA encoding the receptor protein of the present invention.

SEQ ID NO:5

55 The amino acid sequence of the receptor protein of the present invention.

SEQ ID NO:6

The base sequence of cDNA from frog embryo encoding eight amino acid sequences characteristic of the receptor

protein of the present invention.

SEQ ID NO:7

5 The amino acid sequence of eight amino acid sequences characteristic of the receptor protein of the present invention.

SEQ ID NO:8

10 The base sequence of a DNA fragment amplified from mouse embryonal carcinoma cell line P19 undergoing nerve induction with retinoic acid by the RT-PCR method using the synthetic DNA of SEQ ID NO:1 and SEQ ID NO:2.

SEQ ID NO:9

15 The base sequence of a synthetic DNA primer used to amplify cDNA encoding the receptor protein of the present invention by the RT-PCR method.

SEQ ID NO:10

20 The base sequence of a synthetic DNA primer used to amplify cDNA encoding the receptor protein of the present invention by the RT-PCR method.

#### Examples

25 The present invention is hereinafter described in more detail by means of the following examples, which are not to be construed as limitative to the present invention.

#### Example 1

30 Preparation of poly(A)<sup>+</sup> RNA fraction from mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid

Mouse embryonal carcinoma cell line ECP19 cells, 10<sup>5</sup> cells/ml, were inoculated to an  $\alpha$ -minimum essential medium containing 10% bovine serum in dishes for *Escherichia coli* cultivation. After retinoic acid (all-trans, produced by Sigma Corporation) was added at  $5 \times 10^{-7}$  M, the cells were subjected to suspended culture for 72 hours at 37°C in the presence of 5% CO<sub>2</sub> and 95% air. The cells were then washed with the same medium to remove the retinoic acid, after which they were transferred to a dish for tissue culture, where they were cultured for 3 days. After cultivation, the cells were recovered via centrifugation and treated by the guanidine isothiocyanate method to prepare total RNA [Kaplan, B.B. et al., *Biochem. J.*, 183, 181-184 (1979)] and prepare a poly(A)<sup>+</sup> RNA fraction using an mRNA purification kit (produced by Pharmacia).

#### Example 2

45 cDNA amplification by RT-PCR method using poly(A)<sup>+</sup> RNA from mouse embryonal carcinoma cell line undergoing neuronal differentiation with retinoic acid

Using 1  $\mu$ g of poly(A)<sup>+</sup> RNA prepared from mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid as a template, amplification by RT-PCR was conducted. The reaction was carried out using synthetic DNA primers (SEQ ID NO:1 and SEQ ID NO:2), each 100 pM, and the TaKaRa RNA PCR kit (ver. 2) (produced by Takara Shuzo), as directed in the kit manual. Amplification was achieved in 30 cycles of 95°C  $\times$  30 seconds, 60°C  $\times$  30 seconds and 72°C  $\times$  1.5 minutes using a thermal cycler (Perkin-Elmer). The amplification product was detected by 2.0% agarose gel electrophoresis and ethidium bromide staining.

#### Example 3

55 Subcloning of RT-PCR product in plasmid vector and selection of new candidate receptor clones by decoding of base sequence of cDNA insert

The RT-PCR reaction product obtained in Example 2 was separated using 2.0% low-melting agarose gel; after

being cut out using a razor, the band portion was subjected to thermal melting, phenol extraction and ethanol precipitation to recover the DNA. The DNA recovered was subcloned into a plasmid vector, as directed in the instruction manual of the TA cloning kit (Invitrogen). After the subcloned DNA was introduced to, and transformed in, *Escherichia coli* JM109 competent cells (produced by Takara Shuzo), clones having a cDNA insert were selected in LB agar medium containing ampicillin, IPTG (isopropyl thio- $\beta$ -D-galacto-side) and X-gal (5-bromo-4-chloro-3-indolyl- $\beta$ -D-galacto-side); the clones showing a white color were separated using a sterilized toothpick to yield transformants of *Escherichia coli* JM109. Each clone was cultured overnight in LB medium containing ampicillin, after which plasmid DNA was prepared using an automatic plasmid extractor (produced by Kurabo). Using a portion of the thus-prepared DNA, Eco RI cleavage was conducted to confirm the size of the cDNA insert. A portion of the remaining DNA was further subjected to RNase treatment and phenol/chloroform extraction, followed by concentration by ethanol precipitation; the resulting concentrate was subjected to base sequencing as described below.

Base sequencing was achieved by reaction using a Dye Deoxy Terminator Cycle Sequencing Kit (produced by ABI) and subsequent decoding using a fluorescent automatic sequencer. The base sequence information obtained was analyzed using DNASIS (produced by Hitachi System Engineering).

The base sequence determined is shown by SEQ ID NO:8.

Homology search based on the base sequence determined demonstrated that the cDNA fragment inserted in the plasmid harbored by the transformant of *Escherichia coli* JM109 encoded a new activin receptor protein resulting from insertion of the 24 bp base sequence shown by SEQ ID NO:3 into a known mouse activin receptor.

#### Example 4

Cloning of cDNA containing entire coding region of receptor protein from cDNA library from mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid

Using 10  $\mu$ g of poly(A)<sup>+</sup> RNA prepared from mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid in Example 1, in combination with a random 9-mer primer, first strand cDNA was synthesized as directed in the instruction manual of the cDNA Synthesis System Plus (produced by Amersham), followed by synthesis of second strand cDNA, to yield 269 ng of double-stranded cDNA. To both ends of this double-stranded cDNA, an adaptor was ligated using a cDNA rapid adaptor ligation module (produced by Amersham) as directed in the instruction manual of the module. To 75 ng of this ligated double-stranded cDNA,  $\lambda$ ZAPII vector arm was ligated using a  $\lambda$ ZAPII/Eco RI/CIAI Treated Vector Kit (produced by Toyobo), as directed in the instruction manual of the kit. Of the resulting cDNA library,  $2.1 \times 10^6$  pfu (plaque forming unit) was mixed with *Escherichia coli* XL1-BlueMRF', previously treated with magnesium sulfate, followed by incubation at 37°C for 15 minutes, after which 0.5% agarose LB was added, and the mixture was sown on 1.5% agar (produced by Wako Pure Chemical Industries) LB plates. On the plates showing plate formation, a nitrocellulose filter was placed to transcribe the plaques thereto. This filter was denatured by alkali treatment, after which it was heated at 80°C for 3 hours to fix the DNA.

This filter was incubated with the following probe at 42°C overnight in a buffer containing 50% formamide, 5  $\times$  SSPE (SSPE: 150 mM NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub>  $\cdot$  H<sub>2</sub>O, 1.25 mM EDTA, pH 7.4), 5  $\times$  Denhardt's solution, 0.1% SDS (sodium dodecyl sulfate) and 100  $\mu$ g/ml salmon sperm DNA to cause hybridization. The probe used was prepared by cleaving the DNA fragment (inserted in a plasmid) obtained in Example 3 with Eco RI and recovering the fragment, and was used after labeling by incorporation of [<sup>32</sup>P] dCTP (produced by DuPont) using a random prime DNA labeling kit (produced by Amersham). The filter was washed with 2  $\times$  SSC (SSC: 150 mM NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub>  $\cdot$  H<sub>2</sub>O) and 0.1% SDS at room temperature for 1 hour and 15 minutes then at 60°C for 1 hour and 15 minutes, followed by autoradiography at -80°C to detect hybridizable plaques.

This screening detected 30 independent plaques showing a hybridization signal. Each plaque was picked up and transferred to 5 ml of SM (50 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 7 mM MgSO<sub>4</sub>, 0.01% gelatin); after vigorous stirring, a portion of the mixture was mixed with *Escherichia coli* XL1-BlueMRF', previously treated with magnesium sulfate, followed by incubation at 37°C for 15 minutes, after which 0.5% agarose (produced by Pharmacia) LB was added, and the mixture was sown on 1.5% agar (produced by Wako Pure Chemical Industries) LB plates. On these plates, a nylon filter was placed to transcribe the plaques thereto. This filter was denatured by alkali treatment in the same manner as above, after which it was heated at 80°C for 3 hours to fix the DNA.

These filters were hybridized to the same probe in the same manner as those described above and washed in the same manner as that described above to detect hybridizable plaques.

This screening demonstrated that 28 clones were positive. To determine whether these clones encode a known activin receptor protein or the new receptor protein of the present invention, each plaque was suspended in distilled water to yield template DNA. Using this template DNA in combination with the synthetic DNA primers shown by SEQ ID NO:1 and SEQ ID NO:2, the PCR method was conducted to confirm the length of amplified DNA fragments.

This screening demonstrated that three clones encode the new receptor protein.

The insert of this clone was then cut out using Eco RI; the resulting fragment was subcloned into the Eco RI site of

pUC118, after which *Escherichia coli* JM109 was transformed with this plasmid to yield a transformant. The base sequence of the cDNA fragment inserted in the plasmid was then determined. Specifically, using the restriction enzyme sites in the Eco RI fragment, unnecessary portions were removed or a necessary fragment was subcloned to yield a template plasmid for base sequencing.

Base sequencing was achieved by reaction using a Dye Deoxy Terminator Cycle Sequencing Kit (produced by ABI) and subsequent decoding using a fluorescent DNA sequencer (produced by ABI). Data analysis was conducted using DNASIS (produced by Hitachi Software Engineering).

The base sequence determined is shown in Figures 3 and 4. This base sequence agreed with the mouse activin receptor type IIA described in Cell, Vol. 65, pp. 973-982 (1991), except for the base sequence shown by SEQ ID NO:3, demonstrating its identity as a new subtype of mouse activin receptor type IIA.

#### Example 5

Detection of expression by the RT-PCR method using poly(A)<sup>+</sup> RNA from various mouse organs

Using 1 µg of poly(A)<sup>+</sup> RNA prepared from mouse ovary, testes, total embryo, lung, heart, liver, skeletal muscle or brain as a template, amplification by RT-PCR was conducted. The reaction was carried out using synthetic DNA primers (SEQ ID NO:1 and SEQ ID NO:2), each 100 pM, and the TaKaRa RNA PCR kit (ver. 2) (produced by Takara Shuzo), as directed in the kit manual. Amplification was achieved in 30 cycles of 95°C × 30 seconds, 60°C × 30 seconds and 72°C × 1.5 minutes using a thermal cycler (produced by Perkin-Elmer). The amplification product was detected by 2.0% agarose gel electrophoresis and ethidium bromide staining. The results are shown in Figure 7. The receptor of the present invention was found to be expressed specifically in the brain, as with the mouse embryonal carcinoma cell line P19 undergoing neuronal differentiation with retinoic acid, demonstrating that the receptor of the present invention is expressed specifically in the nervous system.

#### Example 6

cDNA amplification by RT-PCR method using poly(A)<sup>+</sup> RNA from human neuroblastoma cell line or frog embryo

Using 1 µg of commercially available poly(A)<sup>+</sup> RNA (produced by Toyo Bouseki) from human neuroblastoma or poly(A)<sup>+</sup> RNA from frog embryo as a template, amplification by RT-PCR was conducted. The reaction was carried out using synthetic DNA primers (SEQ ID NO:1 and SEQ ID NO:2 for the human RNA; SEQ ID NO:9 and SEQ ID NO:10 for the frog RNA), each 100 pM, and the TaKaRa RNA PCR kit (ver. 2) (produced by Takara Shuzo), as directed in the kit manual. Amplification was achieved in 30 cycles of 95°C × 30 seconds, 60°C × 30 seconds and 72°C × 1.5 minutes using a thermal cycler (produced by Perkin-Elmer). The amplification product was detected by 2.0% agarose gel electrophoresis and ethidium bromide staining.

#### Example 7

Subcloning of RT-PCR product into plasmid vector and selection of new candidate receptor clones by decoding of base sequence of cDNA insert

The RT-PCR reaction product obtained in Example 6 was separated using 1.0% low-melting agarose gel; after being cut out using a razor, the band portion was subjected to thermal melting, phenol extraction and ethanol precipitation to recover the DNA. The DNA recovered was subcloned into a plasmid vector, as directed in the instruction manual of the TA cloning kit (produced by Invitrogen). After the subcloned DNA was introduced to, and transformed in, *Escherichia coli* JM109 competent cells (produced by Takara Shuzo), clones having a cDNA insert were selected in LB agar medium containing ampicillin, IPTG (isopropyl thio-β-D-galactoside) and X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside); the clones showing a white color were separated using a sterilized toothpick to yield transformants of *Escherichia coli*. Each clone was cultured overnight in LB medium containing ampicillin, after which plasmid DNA was prepared using an automatic plasmid extractor (produced by Kurabo), and subjected to base sequencing as described below.

Base sequencing was achieved by reaction using a Dye Deoxy Terminator Cycle Sequencing Kit (produced by ABI) and subsequent decoding using a fluorescent automatic sequencer. The base sequence information obtained was analyzed using DNASIS (produced by Hitachi System Engineering).

Homology search based on the base sequence determined demonstrated that the cDNA fragment from the human neuroblastoma cell line agreed with SEQ ID NO:3. The amino acid sequence deduced from this base sequence is shown by SEQ ID NO:7.

The base sequence of the cDNA fragment from frog embryo was determined to be SEQ ID NO:6; the amino acid

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sequence deduced from the base sequence agreed with SEQ ID NO:7.

The receptor protein and protein-encoding DNA of the present invention can be used as reagents for various purposes, including 1) ligand determination, 2) obtainment of antibodies and antisera, 3) construction of recombinant receptor protein expression systems, 4) development of receptor binding assay systems and screening for pharmaceutical candidate compounds using such expression systems, 5) drug designing based on comparison with structurally similar ligand receptors, 6) preparation of probes and PCR primers for gene diagnosis, and 7) drugs for gene therapy. Elucidation of the structures and natures of activin receptors, in particular, would lead to the development of unique pharmaceuticals that act on these systems.

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SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Takeda Chemical Industries, Ltd.  
(B) STREET: 1-1, Doshomachi 4-chome, Chuo-ku  
(C) CITY: Osaka-shi  
(D) STATE: Osaka  
(E) COUNTRY: Japan  
(F) POSTAL CODE (ZIP): 541

(ii) TITLE OF INVENTION: Neuronal Cell-Specific Receptor Protein

(iii) NUMBER OF SEQUENCES: 10

15 (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

20 (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TACCCCTCTG TACTGTTC TACTCAA

27

35 (2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TAGCCACAGG TCCACATCCA CACTGGT

27

(2) INFORMATION FOR SEQ ID NO: 3:

50 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: other nucleic acid  
(A) DESCRIPTION: /desc = "synthetic DNA"

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CACGCCTTTC ATATAATGAT AGAG

24

10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2122 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 9..1571

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCGGGAAA ATG GGA GCT GCT GCA AAG TTG GCG TTC GCC GTC TTT CTT ATC  
Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile  
1 5 10

50

25

TCT TGC TCT TCA GGT GCT ATA CTT GGC AGA TCA GAA ACT CAG GAG TGT  
Ser Cys Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys  
15 20 25 30

98

30

CTT TTC TTT AAT GCT AAT TGG GAA AGA GAC AGA ACC AAC CAG ACT GGT  
Leu Phe Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr Gly  
35 40 45

146

GTT GAA CCT TGC TAT GGT GAT AAA GAT AAA CGG CGA CAT TGT TTT GCT  
Val Glu Pro Cys Tyr Gly Asp Lys Asp Lys Arg Arg His Cys Phe Ala  
50 55 60

194

35

ACC TGG AAG AAT ATT TCT GGT TCC ATT GAA ATA GTG AAG CAA GGT TGT  
Thr Trp Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys  
65 70 75

242

TGG CTG GAT GAT ATC AAC TGC TAT GAC AGG ACT GAT TGT ATA GAA AAA  
Trp Leu Asp Asp Ile Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu Lys  
80 85 90

290

40

AAA GAC AGC CCT GAA GTG TAC TTT TGT TGC TGT GAG GGC AAT ATG TGT  
Lys Asp Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys  
95 100 105 110

338

45

AAT GAA AAG TTC TCT TAT TTT CCG GAG ATG GAA GTC ACA CAG CCC ACT  
Asn Glu Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr  
115 120 125

386

TCA AAT CCT GTT ACA CCG AAG CCA CCC TAT TAC AAC ATT CTG CTG TAT  
Ser Asn Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn Ile Leu Leu Tyr  
130 135 140

434

50

TCC TTG GTA CCA CTA ATG TTA ATT GCA GGA ATT GTC ATT TGT GCA TTT  
Ser Leu Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe  
145 150 155

482

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	TGG	GTG	TAC	AGA	CAT	CAC	AAG	ATG	GCC	TAC	CCT	CCT	GTA	CTT	GTT	CCT	530
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				210					215					220			
15	TAT	GTG	GCT	GTC	AAA	ATA	TTT	CCA	ATA	CAG	GAC	AAA	CAG	TCC	TGG	CAG	722
	Tyr	Val	Ala	Val	Lys	Ile	Phe	Pro	Ile	Gln	Asp	Lys	Gln	Ser	Trp	Gln	
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	AAT	GAA	TAT	GAA	GTC	TAT	AGT	CTA	CCT	GGA	ATG	AAG	CAT	GAG	AAC	ATA	770
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	240						245					250					
20	CTA	CAG	TTC	ATT	GGT	GCA	GAG	AAA	AGA	GGC	ACC	AGT	GTG	GAT	GTG	GAC	818
	Leu	Gln	Phe	Ile	Gly	Ala	Glu	Lys	Arg	Gly	Thr	Ser	Val	Asp	Val	Asp	
	255					260					265					270	
	CTG	TGG	CTA	ATC	ACA	GCA	TTT	CAT	GAA	AAG	GGC	TCA	CTG	TCA	GAC	TTT	866
	Leu	Trp	Leu	Ile	Thr	Ala	Phe	His	Glu	Lys	Gly	Ser	Leu	Ser	Asp	Phe	
25					275					280					285		
	CTT	AAG	GCT	AAT	GTG	GTC	TCT	TGG	AAT	GAA	CTT	TGT	CAT	ATT	GCA	GAA	914
	Leu	Lys	Ala	Asn	Val	Val	Ser	Trp	Asn	Glu	Leu	Cys	His	Ile	Ala	Glu	
				290					295					300			
30	ACC	ATG	GCT	AGA	GGA	TTG	GCA	TAT	TTA	CAT	GAG	GAT	ATA	CCT	GGC	TTA	962
	Thr	Met	Ala	Arg	Gly	Leu	Ala	Tyr	Leu	His	Glu	Asp	Ile	Pro	Gly	Leu	
			305					310					315				
	AAA	GAT	GGC	CAC	AAG	CCT	GCA	ATC	TCT	CAC	AGG	GAC	ATC	AAA	AGT	AAA	1010
	Lys	Asp	Gly	His	Lys	Pro	Ala	Ile	Ser	His	Arg	Asp	Ile	Lys	Ser	Lys	
	320					325						330					
35	AAT	GTG	CTG	TTG	AAA	AAC	AAT	CTG	ACA	GCT	TGC	ATT	GCT	GAC	TTT	GGG	1058
	Asn	Val	Leu	Leu	Lys	Asn	Asn	Leu	Thr	Ala	Cys	Ile	Ala	Asp	Phe	Gly	
	335					340					345					350	
	TTG	GCC	TTA	AAG	TTC	GAG	GCT	GGC	AAG	TCT	GCA	GGT	GAC	ACC	CAT	GGG	1106
	Leu	Ala	Leu	Lys	Phe	Glu	Ala	Gly	Lys	Ser	Ala	Gly	Asp	Thr	His	Gly	
40					355					360					365		
	CAG	GTT	GGT	ACC	CGG	AGG	TAT	ATG	GCT	CCA	GAG	GTG	TTG	GAG	GGT	GCT	1154
	Gln	Val	Gly	Thr	Arg	Arg	Tyr	Met	Ala	Pro	Glu	Val	Leu	Glu	Gly	Ala	
				370					375					380			
45	ATA	AAC	TTC	CAA	AGG	GAC	GCA	TTT	CTG	AGG	ATA	GAT	ATG	TAC	GCC	ATG	1202
	Ile	Asn	Phe	Gln	Arg	Asp	Ala	Phe	Leu	Arg	Ile	Asp	Met	Tyr	Ala	Met	
			385					390					395				
	GGA	TTA	GTC	CTA	TGG	GAA	TTG	GCT	TCT	CGT	TGC	ACT	GCT	GCA	GAT	GGA	1250
	Gly	Leu	Val	Leu	Trp	Glu	Leu	Ala	Ser	Arg	Cys	Thr	Ala	Ala	Asp	Gly	
	400					405						410					
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	415					420					425					430	

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CCA TCT CTT GAA GAT ATG CAG GAA GTT GTT GTG CAT AAA AAA AAG AGG 1346  
Pro Ser Leu Glu Asp Met Gln Glu Val Val Val His Lys Lys Lys Arg  
435 440 445

5 CCT GTT TTA AGA GAT TAT TGG CAG AAA CAT GCA GGA ATG GCA ATG CTC 1394  
Pro Val Leu Arg Asp Tyr Trp Gln Lys His Ala Gly Met Ala Met Leu  
450 455 460

TGT GAA ACG ATA GAA GAA TGT TGG GAT CAT GAT GCA GAA GCC AGG TTA 1442  
Cys Glu Thr Ile Glu Glu Cys Trp Asp His Asp Ala Glu Ala Arg Leu  
465 470 475

10 TCA GCT GGA TGT GTA GGT GAA AGA ATT ACT CAG ATG CAA AGA CTA ACA 1490  
Ser Ala Gly Cys Val Gly Glu Arg Ile Thr Gln Met Gln Arg Leu Thr  
480 485 490

AAT ATC ATT ACT ACA GAG GAC ATT GTA ACA GTG GTC ACA ATG GTG ACA 1538  
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495 500 505 510

15 AAT GTT GAC TTT CCT CCC AAA GAA TCT AGT CTA TGATGGTGGC ACCGTCCTGTA 1591  
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515 520

20 CACACTGAGG ACTGGGACTC TGAAGTGGAG CTGCTAAGCT AAGGAAAGTG CTTAGTTGAT 1651

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AGCATGGATC TGGGAGATGG ATCTGGGAAA CTTACTGCAT CGTCTGCAGC ACAGATATGA 1771

25 AGAGGAGTCT AAGGGAAAAG CTGCAAACTG TAAAGAACTT CTGAAAATGT ACTCGAAGAA 1831

TGTGGCCCTC TCCAAATCAA GGATCTTTTG GACCTGGCTA ATCAAGTATT TGCAAACTG 1891

ACATCAGATT TCTTAATGTC TGTCAGAAGA CACTAATTCC TTAAATGAAC TACTGCTATT 1951

TTTTTTAAAT GAAAACTTT TCATTTTCTG TTTTAAAAAG GGTAACTTTT TATTGCATTT 2011

30 GCTGTTGTTT CTATAAATGA CTATTGTAAT GCCAACATGA CACAGCTTGT GAATGTGTAG 2071

TGTGCTGCTG TTCTGTGTAC ATAGTCATCA AAGTGGGGTA CAGTAAAGAG G 2122

## (2) INFORMATION FOR SEQ ID NO: 5:

35

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 521 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

40

- (ii) MOLECULE TYPE: protein  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys  
1 5 10 15

Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe  
20 25 30

45 Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr Gly Val Glu  
35 40 45

Pro Cys Tyr Gly Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp  
50 55 60

50 Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu  
65 70 75 80

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Asp Asp Ile Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu Lys Lys Asp  
85 90 95

5 Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu  
100 105 110

Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn  
115 120 125

10 Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn Ile Leu Leu Tyr Ser Leu  
130 135 140

Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe Trp Val  
145 150 155 160

15 Tyr Arg His His Lys Met Ala Tyr Pro Pro Val Leu Val Pro Thr Gln  
165 170 175

His Ala Phe His Ile Met Ile Glu Asp Pro Gly Pro Pro Pro Ser  
180 185 190

20 Pro Leu Leu Gly Leu Lys Pro Leu Gln Leu Leu Glu Val Lys Ala Arg  
195 200 205

Gly Arg Phe Gly Cys Val Trp Lys Ala Gln Leu Leu Asn Glu Tyr Val  
210 215 220

25 Ala Val Lys Ile Phe Pro Ile Gln Asp Lys Gln Ser Trp Gln Asn Glu  
225 230 235 240

Tyr Glu Val Tyr Ser Leu Pro Gly Met Lys His Glu Asn Ile Leu Gln  
245 250 255

Phe Ile Gly Ala Glu Lys Arg Gly Thr Ser Val Asp Val Asp Leu Trp  
260 265 270

30 Leu Ile Thr Ala Phe His Glu Lys Gly Ser Leu Ser Asp Phe Leu Lys  
275 280 285

Ala Asn Val Val Ser Trp Asn Glu Leu Cys His Ile Ala Glu Thr Met  
290 295 300

35 Ala Arg Gly Leu Ala Tyr Leu His Glu Asp Ile Pro Gly Leu Lys Asp  
305 310 315 320

Gly His Lys Pro Ala Ile Ser His Arg Asp Ile Lys Ser Lys Asn Val  
325 330 335

40 Leu Leu Lys Asn Asn Leu Thr Ala Cys Ile Ala Asp Phe Gly Leu Ala  
340 345 350

Leu Lys Phe Glu Ala Gly Lys Ser Ala Gly Asp Thr His Gly Gln Val  
355 360 365

45 Gly Thr Arg Arg Tyr Met Ala Pro Glu Val Leu Glu Gly Ala Ile Asn  
370 375 380

Phe Gln Arg Asp Ala Phe Leu Arg Ile Asp Met Tyr Ala Met Gly Leu  
385 390 395 400

50 Val Leu Trp Glu Leu Ala Ser Arg Cys Thr Ala Ala Asp Gly Pro Val  
405 410 415

Asp Glu Tyr Met Leu Pro Phe Glu Glu Glu Ile Gly Gln His Pro Ser  
420 425 430

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Leu Glu Asp Met Gln Glu Val Val Val His Lys Lys Lys Arg Pro Val  
 435 440 445  
 5 Leu Arg Asp Tyr Trp Gln Lys His Ala Gly Met Ala Met Leu Cys Glu  
 450 455 460  
 Thr Ile Glu Glu Cys Trp Asp His Asp Ala Glu Ala Arg Leu Ser Ala  
 465 470 475 480  
 10 Gly Cys Val Gly Glu Arg Ile Thr Gln Met Gln Arg Leu Thr Asn Ile  
 485 490 495  
 Ile Thr Thr Glu Asp Ile Val Thr Val Val Thr Met Val Thr Asn Val  
 500 505 510  
 15 Asp Phe Pro Pro Lys Glu Ser Ser Leu  
 515 520

## (2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CACGCCTTTC ACATTATGAT AGAG

24

## (2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 8 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

His Ala Phe His Ile Met Ile Glu  
 1 5

## (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 294 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

5 TACCCTCCTG TACTTGTTCC TACTCAAGAC CCAGGACCAC CCCCACCTTC CCCATTACTA 60  
GGGTTGAAGC CATTGCAGCT GTTAGAAGTG AAAGCAAGGG GAAGATTTCG TTGTGTCTGG 120  
AAAGCCCAGT TGCTCAATGA ATATGTGGCT GTCAAAATAT TTCCAATACA GGACAAACAG 180  
TCCTGGCAGA ATGAATATGA AGTCTATAGT CTACCTGGAA TGAAGCATGA GAACATACTA 240  
10 CAGTTCATTG GTGCAGAGAA AAGAGGCACC AGTGTGGATG TGGACCTGTG GCTA 294

(2) INFORMATION FOR SEQ ID NO: 9:

15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
20 (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

25 TCGCCTACCC CCCAGTGCT 19

(2) INFORMATION FOR SEQ ID NO: 10:

30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(ii) MOLECULE TYPE: other nucleic acid  
35 (A) DESCRIPTION: /desc = "synthetic DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

40 TCTGTGTCAA GGTGTGT 17

#### 45 Claims

1. A receptor protein containing an amino acid sequence substantially shown by the formula:  
His Ala Phe His Ile Met Ile Glu  
50 or a salt thereof.
2. The protein of claim 1 wherein said receptor protein is a neuronal cell-specific receptor protein.
3. The protein of claim 2 wherein said receptor protein is an activin receptor protein.
- 55 4. A receptor protein containing or comprising an amino acid sequence shown by  
X- Gly Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln  
Glu Cys Leu Phe Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly Asp Lys Asp  
Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile

Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu Lys Lys Asp Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys  
 Asn Glu Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn  
 Ile Leu Leu Tyr Ser Leu Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe Trp Val Tyr Arg His His Lys Met Ala  
 Tyr Pro Pro Val Leu Val Pro Thr Gln His Ala Phe His Ile Met Ile Glu Asp Pro Gly Pro Pro Pro Pro Ser Pro Leu Leu  
 5 Gly Leu Lys Pro Leu Gln Leu Leu Glu Val Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln Leu Leu Asn Glu  
 Tyr Val Ala Val Lys Ile Phe Pro Ile Gln Asp Lys Gln Ser Trp Gln Asn Glu Tyr Glu Val Tyr Ser Leu Pro Gly Met Lys His  
 Glu Asn Ile Leu Gln Phe Ile Gly Ala Glu Lys Arg Gly Thr Ser Val Asp Val Asp Leu Trp Leu Ile Thr Ala Phe His Glu  
 Lys Gly Ser Leu Ser Asp Phe Leu Lys Ala Asn Val Val Ser Trp Asn Glu Leu Cys His Ile Ala Glu Thr Met Ala Arg Gly  
 Leu Ala Tyr Leu His Glu Asp Ile Pro Gly Leu Lys Asp Gly His Lys Pro Ala Ile Ser His Arg Asp Ile Lys Ser Lys Asn  
 10 Val Leu Leu Lys Asn Asn Leu Thr Ala Cys Ile Ala Asp Phe Gly Leu Ala Leu Lys Phe Glu Ala Gly Lys Ser Ala Gly  
 Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe  
 Leu Arg Ile Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Ala Ser Arg Cys Thr Ala Ala Asp Gly Pro Val Asp Glu  
 Tyr Met Leu Pro Phe Glu Glu Glu Ile Gly Gln His Pro Ser Leu Glu Asp Met Gln Glu Val Val Val His Lys Lys Lys Arg  
 Pro Val Leu Arg Asp Tyr Trp Gln Lys His Ala Gly Met Ala Met Leu Cys Glu Thr Ile Glu Glu Cys Trp Asp His Asp Ala  
 15 Glu Ala Arg Leu Ser Ala Gly Cys Val Gly Glu Arg Ile Thr Gln Met Gln Arg Leu Thr Asn Ile Ile Thr Thr Glu Asp Ile Val  
 Thr Val Val Thr Met Val Thr Asn Val Asp Phe Pro Pro Lys Glu Ser Ser Leu,  
 wherein X represents Met that may be protected or hydrogen, or any portion thereof which also contains the amino  
 acid sequence substantially shown by the formula: His Ala Phe His Ile Met Ile Glu, or a salt thereof.

- 20 5. The protein of claim 3 having an amino acid sequence shown by  
 X- Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln  
 Glu Cys Leu Phe Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr Gly Val Glu Pro Cys Tyr Gly Asp Lys Asp  
 Lys Arg Arg His Cys Phe Ala Thr Trp Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu Asp Asp Ile  
 25 Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu Lys Lys Asp Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys  
 Asn Glu Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn  
 Ile Leu Leu Tyr Ser Leu Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe Trp Val Tyr Arg His His Lys Met Ala  
 Tyr Pro Pro Val Leu Val Pro Thr Gln His Ala Phe His Ile Met Ile Glu Asp Pro Gly Pro Pro Pro Pro Set Pro Leu Leu  
 Gly Leu Lys Pro Leu Gln Leu Leu Glu Val Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Gln Leu Leu Asn Glu  
 Tyr Val Ala Val Lys Ile Phe Pro Ile Gln Asp Lys Gln Ser Trp Gln Asn Glu Tyr Glu Val Tyr Ser Leu Pro Gly Met Lys His  
 30 Glu Asn Ile Leu Gln Phe Ile Gly Ala Glu Lys Arg Gly Thr Ser Val Asp Val Asp Leu Trp Leu Ile Thr Ala Phe His Glu  
 Lys Gly Ser Leu Ser Asp Phe Leu Lys Ala Asn Val Val Ser Trp Asn Glu Leu Cys His Ile Ala Glu Thr Met Ala Arg Gly  
 Leu Ala Tyr Leu His Glu Asp Ile Pro Gly Leu Lys Asp Gly His Lys Pro Ala Ile Ser His Arg Asp Ile Lys Ser Lys Asn  
 Val Leu Leu Lys Asn Asn Leu Thr Ala Cys Ile Ala Asp Phe Gly Leu Ala Leu Lys Phe Glu Ala Gly Lys Ser Ala Gly  
 Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe  
 35 Leu Arg Ile Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Ala Ser Arg Cys Thr Ala Ala Asp Gly Pro Val Asp Glu  
 Tyr Met Leu Pro Phe Glu Glu Glu Ile Gly Gln His Pro Ser Leu Glu Asp Met Gln Glu Val Val Val His Lys Lys Lys Arg  
 Pro Val Leu Arg Asp Tyr Trp Gln Lys His Ala Gly Met Ala Met Leu Cys Glu Thr Ile Glu Glu Cys Trp Asp His Asp Ala  
 Glu Ala Arg Leu Ser Ala Gly Cys Val Gly Glu Arg Ile Thr Gln Met Gln Arg Leu Thr Asn Ile Ile Thr Thr Glu Asp Ile Val  
 Thr Val Val Thr Met Val Thr Asn Val Asp Phe Pro Pro Lys Glu Ser Ser Leu,  
 40 wherein X represents Met that may be protected or hydrogen; or a salt thereof.
6. A protein containing an amino acid sequence substantially comprised of amino acid residues His Ala Phe His Ile  
 Met Ile Glu, wherein said protein is capable of binding with activin or activin like substances or a portion thereof.
- 45 7. An isolated and purified DNA sequence encoding a protein containing an amino acid sequence sufficiently dupli-  
 cative of the sequence His Ala Phe His Ile Met Ile Glu to allow possession of the biological property of binding with  
 activin or activin containing substances.
8. A DNA containing a nucleotide sequence encoding the protein of claim 1.
- 50 9. The DNA of claim 8, containing a nucleotide sequence shown by  
 X' GGA GCT GCT GCA AAG TTG GCG TTC GCC GTC TTT CTT ATC TCT TGC TCT TCA GGT GCT ATA CTT  
 GGC AGA TCA GAA ACT CAG GAG TGT CTT TTC TTT AAT GCT AAT TGG GAA AGA GAC AGA ACC AAC CAG  
 ACT GGT GTT GAA CCT TGC TAT GGT GAT AAA GAT AAA CGG CGA CAT TGT TTT GCT ACC TGG AAG AAT  
 55 ATT TCT GGT TCC ATT GAA ATA GTG AAG CAA GGT TGT TGG CTG GAT GAT ATC AAC TGC TAT GAC AGG  
 ACT GAT TGT ATA GAA AAA AAA GAC AGC CCT GAA GTG TAC TTT TGT TGC TGT GAG GGC AAT ATG TGT  
 AAT GAA AAG TTC TCT TAT TTT CCG GAG ATG GAA GTC ACA CAG CCC ACT TCA AAT CCT GTT ACA CCG  
 AAG CCA CCC TAT TAC AAC ATT CTG CTG TAT TCC TTG GTA CCA CTA ATG TTA ATT GCA GGA ATT GTC  
 ATT TGT GCA TTT TGG GTG TAC AGA CAT CAC AAG ATG GCC TAC CCT CCT GTA CTT GTT CCT ACT CAA

CAC GCC TTT CAT ATA ATG ATA GAG GAC CCA GGA CCA CCC CCA CCT TCC CCA TTA CTA GGG TTG AAG  
 CCA TTG CAG CTG TTA GAA GTG AAA GCA AGG GGA AGA TTT GGT TGT GTC TGG AAA GCC CAG TTG  
 CTC AAT GAA TAT GTG GCT GTC AAA ATA TTT CCA ATA CAG GAC AAA CAG TCC TGG CAG AAT GAA TAT  
 GAA GTC TAT AGT CTA CCT GGA ATG AAG CAT GAG AAC ATA CTA CAG TTC ATT GGT GCA GAG AAA AGA  
 5 GGC ACC AGT GTG GAT GTG GAC CTG TGG CTA ATC ACA GCA TTT CAT GAA AAG GGC TCA CTG TCA  
 GAC TTT CTT AAG GCT AAT GTG GTC TCT TGG AAT GAA CTT TGT CAT ATT GCA GAA ACC ATG GCT AGA  
 GGA TTG GCA TAT TTA CAT GAG GAT ATA CCT GGC TTA AAA GAT GGC CAC AAG CCT GCA ATC TCT CAC  
 AGG GAC ATC AAA AGT AAA AAT GTG CTG TTG AAA AAC AAT CTG ACA GCT TGC ATT GCT GAC TTT GGG  
 TTG GCC TTA AAG TTC GAG GCT GGC AAG TCT GCA GGT GAC ACC CAT GGG CAG GTT GGT ACC CGG  
 10 AGG TAT ATG GCT CCA GAG GTG TTG GAG GGT GCT ATA AAC TTC CAA AGG GAC GCA TTT CTG AGG ATA  
 GAT ATG TAC GCC ATG GGA TTA GTC CTA TGG GAA TTG GCT TCT CGT TGC ACT GCT GCA GAT GGA CCC  
 GTA GAT GAG TAC ATG TTA CCA TTT GAG GAA GAA ATT GGC CAG CAT CCA TCT CTT GAA GAT ATG CAG  
 GAA GTT GTT GTG CAT AAA AAA AAG AGG CCT GTT TTA AGA GAT TAT TGG CAG AAA CAT GCA GGA ATG  
 GCA ATG CTC TGT GAA ACG ATA GAA GAA TGT TGG GAT CAT GAT GCA GAA GCC AGG TTA TCA GCT GGA  
 15 TGT GTA GGT GAA AGA ATT ACT CAG ATG CAA AGA CTA ACA AAT ATC ATT ACT ACA GAG GAC ATT GTA  
 ACA GTG GTC ACA ATG GTG ACA AAT GTT GAC TTT CCT CCC AAA GAA TCT AGT CTA,  
 wherein X' represents ATG or hydrogen.

10. A vector containing the DNA of claim 7.
- 20 11. A transformant harboring the vector of claim 10.
12. A method of producing the protein of claim 1 by culturing the transformant of claim 11 to produce and accumulate the protein or a salt thereof in the culture, and collecting said protein.
- 25 13. A method of producing the protein of claim 1 by culturing the transformant of claim 11 to produce and accumulate the protein or a salt thereof in the culture supernatant or on the transformant's cell membrane, and collecting said protein.
- 30 14. A method of detecting the DNA of claim 8 characterized by conducting the polymerase chain reaction method using synthetic DNA containing the base sequence of the formula:  
 TACCCTCCTGTACTTGTCTACTCAA  
 and the formula:  
 TAGCCACAGGTCCACATCCACACTGGT.
- 35 15. An antibody against the protein of claim 1.
16. A method of quantifying the protein of claim 1 by competitively reacting a subject solution containing the protein of claim 1 and the labeled protein of claim 1 to the antibody of claim 15.
- 40 17. A method of determining a ligand against the protein of claim 1, wherein the protein of claim 1 is brought into contact with a test compound.
18. A method of screening for a compound that inhibits or promotes the binding of the protein of claim 1 with a ligand wherein comparison is made between the binding affinities achieved in case (i) in which the ligand is brought into contact with the protein of claim 1, and case (ii) in which both the ligand and the compound are brought into contact with the protein of claim 1.
- 45 19. A screening kit containing the protein of claim 1 and a ligand against the protein of claim 1, which screens for a compound that inhibits or promotes (i) the binding of a ligand against the protein of claim 1 or (ii) the binding of a ligand against a known activin receptor protein or a salt thereof.
- 50 20. An activin receptor agonist or activin receptor antagonist obtained by using the screening method of claim 18 and/or the screening kit of claim 19.
- 55 21. A diagnostic method for nerve degeneration disease wherein the DNA detection method of claim 14 is used.



Figure 1

1 CTCCGAGGAGACCCAGGAACTGGATATCTACCGAGAACTTCTACGGCTTCTCCGGCCCTCGGAAATGGGAGCTGCTGCAAAGTT 90  
 -19 M G A A A K L -13  
 91 GCGTTCGCCGCTCTTCTATCTCTTCAGGTGCTATACCTTGGCAGATCAGAACTCAGGAGTGTCTTTCTTTAATGCTAATTG 180  
 -12 A F A V F L I S C S S G A I L G R S E T Q E C L F F N A N W 18  
 181 GGAAGAGACAGAACCAACCAGACTGGTGTGAACCTTGGCTATGGTGATAAAGATAACGGCGACATTTGTTGCTACCTGGAAGAATAT 270  
 19 E R D R T N Q T G V E P C Y G D K R R H C F A T W K N I 48  
 271 TTCTGGTTCATTGAATAGTGAAGCAAGTTGTTGGCTGGATGATATCAACTGCTATGACAGGACTGATTGTATAGAAAAAAGACAG 360  
 49 S G S I E I V K Q G C W L D D I N C Y D R T D C I E K K D S 78  
 361 CCCTGAAGTGTAATTTGTTGCTGTGAGGGCAATATGTGTAATGAAAGTTCTCTTATTTCCGGAGATGGAAGTCACACAGCCCACTTC 450  
 79 P E V Y F C C C E G N M C N E K F S Y F P E M E V T Q P T S 108  
 451 AAATCCTGTTACACCGAAGCCACCTATTACAAACATTTCTGCTGATTCCTTGCTACCACTAATGTTAATTGCAGGAATTGTCTATTGTGC 540  
 109 N P V T P K P Y Y N I L L Y S L V P L M L I A G I V I C A 138  
 541 ATTTGGGTGTACAGACATCACAGATGGCCTACCTCTGCTACTTGTTCCTACTCAAGACCCAGACCCCACTTCCCCATTACT 630  
 139 F W V Y R H H K M A Y P P V L V P T Q D P G P P P S P L L 168  
 631 AGGTTGAAGCCATTGCACCTGTTAGAAGTGAAGCAAGGGGAAGATTGTTGTTGCTGGAAGCCCACTTGTCTCAATGAATATGTGTC 720  
 169 G L K P L Q L L E V K A R G R F G C V W K A Q L L N E Y V A 198  
 721 TGCAAAATATTTCCAATACAGGACAAACAGTCCTGGCAGAAATGAATATGAAGTCTATAGTCTACCTGGAATGAAGCATGAGAATACAT 810  
 199 V K I F P I Q D K Q S W Q N E Y E V Y S L P G M K H E N I L 228  
 811 ACAGTTCAATTGGTGCAGAGAAAAGAGCCAGCTGTGGATGTGACCTGTGGCTAATCAGACAGATTTTCATCAAAAAGGCTCACCTGCAGA 900  
 229 Q F I G A E K R G T S V D V D L W L I T A F H E K G S L S D 258  
 901 CTTTCTTAAGGCTAATGGTCTCTTGGAAATGAACCTTGTGCATATTGCAGAAACCATGGCTAGAGGATTGGCATATTTACATGAGGATAT 990  
 259 F L K A N V V S W N E L C H I A E T M A R G L A Y L H E D I 288

Figure 2

991 ACCTGGCTTAAAGATGGCCACAAAGCCTGCAATCTCTCACAGGGACATCAAAAGTAAATGTGCTGTGAAAACAATCTGACAGCTTG 1080  
 289 P G L K D G H K P A I S H R D I K S K N V L L K N N L T A C 318  
 1081 CATTCGTGACTTTGGGTTGGCCTTAAAGTTCGAGCTGGCAAGTCTGCAGGTCAACACCCATGGCGAGGTTGGTACCCGAGGTATATGGC 1170  
 319 I A D F G L A L K F E A G K S A G D T H G Q V G T R R Y M A 348  
 1171 TCCAGAGCTGTTGAGGGTGTCTATAAATTCCAAAGGGACCCATTTCTGAGGATAGATATGTACGCCATGGGATTAGTCTTATGGGAATT 1260  
 349 P E V L E G A I N F Q R D A F L R I D M Y A M G L V L W E L 378  
 1261 GCGTCTCGTTCGACTGCTGCAGATGGACCCGTAGATGAGTACATGTTACCATTGAGGAAGAAATGGCCAGCATCCATCTCTTGAAGA 1350  
 379 A S R C T A A D G P V D E Y M L P F E E I G Q H P S L E D 408  
 1351 TATGACGAAGTTGTTGTGCATATAAAAGAGGCCCTGTTTAAAGAGATTATTGGCAGAAACATGCAGGAATGGCAATGCTCTGTGAAAC 1440  
 409 M Q E V V V H K K K R P V L R D Y W Q K H A G M A M L C E T 438  
 1441 GATAGAAGAAATGTTGGGATCATGATGCAGAACGCCAGTTATCAGCTGGATGTGTAGTGAAGAAATTAATCTCAGATGCAAGAGACTAACAAA 1530  
 439 I E E C W D H D A E A R L S A G C V G E R I T Q M Q R L T N 468  
 1531 TATCATTAACAGAGGACATTGTAAACAGTGTGTCAATGGTGACAAATGTTGACTTTCCTCCCAAAGAAATCTAGTCTATGATGGTGCCA 1620  
 469 I I T T E D I V T V V T M V T N V D F P P K E S S L \* 494  
 1621 CCGTCTGTACACACTGAGACTGGGACTCTGAACTGGAGCTGCTAAGCTAAGGAAAGTGCTTACTGTTGTTCTGTGAAATGAGTAGG 1710  
 1711 ATGCTCCAGGACATGTACGCAAGCAGCCCTTGTGGAAGCATGGATCTGGGAGATGGATCTGGGAAACTTACTGCCATCGTCTGCAGCA 1800  
 1801 CAGATATGAGAGGAGTCTAAGGGAAGAGTGCACAACTGTAAGAATGCTAAGAAATGCTGCGAAAGATGTGGCCCTCTCCAAATCAAG 1890  
 1891 GATCTTTGGACCTGGTAATCAAGTATTTCGCAAACTGACATCAGATTTCTTAATGCTGTGCAAGAGACACTAAATTCCTTAAATGAACT 1980  
 1981 ACTGCTATTTTAAATGAAAACCTTTTCATTTTCAGATTTTAAAGGGTAACTTTTATTGTCATTTGCTGTTGTTCTTAAATGAC 2070  
 2071 TATTGTAATGCCAACATGACACAGCTTGTGAATGTGTAGTGTGCTGCTGTGTACATAGTCATCAAGTGGGTACAGTAAAGAGG 2160  
 2161 CTTCCAAGCATTAATTAACCTCCCTCAACAAGGTATACCTCAGTCCACGTTGTTAAATTTAAATTTGAAAACACTAACAGAAATTG 2250  
 2251 AATAAATCAGTCCATGTTTATAACAAGGTTAATTACAAATTCAGTGTGTTATTAAAGAAAATGGTAAAGCTATGCTTAGTGCCAATAG 2340  
 2341 TAATGGCTATTGTAAAGCAGTGTTTTAGCTTTTCTTCTACTGGCTGTAAATTTAGGGAACAAGTGTGCTGCTTTTGAATGGAAGA 2430  
 2431 ATATGGTGTCAACCTACCCCATACCTTATATCAAGTCCCAAAATATTCTTTTCCATTTCAAAGACAGCACTTTTGAAAACCCCTAAATTA 2520  
 2521 CAAGCCAGTAGAAGAAAGCTAAACACCGCTTTTACAAATAGCC 2563



Figure 4

tggcaagtctgcaggtgacacccatgggcaggttggtagccggaggatatggctccaga  
gggtgttgagggtgctataaacttccaaaggacgcatttctgaggatagatatgtacgc  
catgggattagtcctatgggaattggcttctcggtgcactgctgcagatggaccgtaga  
tgagtacatgttaccatttgaggaagaaattggccagcatccatctcttgaagatatgca  
ggaagttgttgtgcataaaaaaagaggcctgttttaagagattattggcagaaacatgc  
aggaatggcaatgctctgtgaaacgatagaagaatgttgggatcatgatgcagaagccag  
gttatcagctggatgtgtaggtgaaagaattactcagatgcaaagactaacaatatcat  
tactacagaggacattgtaacagtggtcacaatggtgacaaatgttgactttcctccaa  
agaatctagtctatgatgggtggcacctgtgtacacactgaggactgggactctgaactg  
gagctgctaagctaaggaaagtgccttagttgattttctgtgtgaaatgagtaggatgcct  
ccaggacatgtacgcaagcagccccctgtggaaagcatggatctgggagatggatctggg  
aaacttactgcatcgtctgcagcacagatatgaagaggagtctaagggaaaagctgcaa  
ctgtaaagaacttctgaaaatgtactcgaagaatgtggccctctccaaatcaaggatctt  
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agacactaattccttaaatgaactactgctatTTTTTTTaaatgaaaaacttttcatttc  
agattttaaaaagggttaactttttattgcatttgcgtgtgtttctataaatgactattgt  
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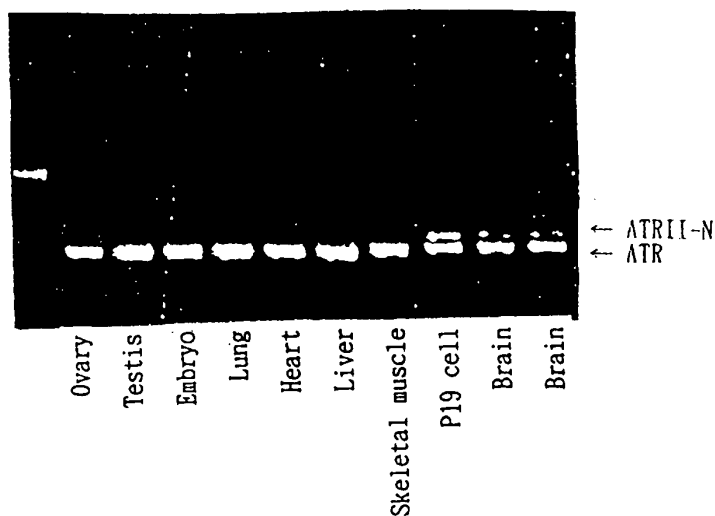
Figure 5

MetGlyAlaAlaAlaLysLeuAlaPheAlaValPheLeuIleSerCysSer  
 SerGlyAlaIleLeuGlyArgSerGluThrGlnGluCysLeuPhePheAsnAlaAsnTrp  
 GluArgAspArgThrAsnGlnThrGlyValGluProCysTyrGlyAspLysAspLysArg  
 ArgHisCysPheAlaThrTrpLysAsnIleSerGlySerIleGluIleValLysGlnGly  
 CysTrpLeuAspAspIleAsnCysTyrAspArgThrAspCysIleGluLysLysAspSer  
 ProGluValTyrPheCysCysCysGluGlyAsnMetCysAsnGluLysPheSerTyrPhe  
 ProGluMetGluValThrGlnProThrSerAsnProValThrProLysProProTyrTyr  
 AsnIleLeuLeuTyrSerLeuValProLeuMetLeuIleAlaGlyIleValIleCysAla  
 PheTrpValTyrArgHisHisLysMetAlaTyrProProValLeuValProThrGlnHis  
 AlaPheHisIleMetIleGluAspProGlyProProProProSerProLeuLeuGlyLeu  
 LysProLeuGlnLeuLeuGluValLysAlaArgGlyArgPheGlyCysValTrpLysAla  
 GlnLeuLeuAsnGluTyrValAlaValLysIlePheProIleGlnAspLysGlnSerTrp  
 GlnAsnGluTyrGluValTyrSerLeuProGlyMetLysHisGluAsnIleLeuGlnPhe  
 IleGlyAlaGluLysArgGlyThrSerValAspValAspLeuTrpLeuIleThrAlaPhe  
 HisGluLysGlySerLeuSerAspPheLeuLysAlaAsnValValSerTrpAsnGluLeu  
 CysHisIleAlaGluThrMetAlaArgGlyLeuAlaTyrLeuHisGluAspIleProGly  
 LeuLysAspGlyHisLysProAlaIleSerHisArgAspIleLysSerLysAsnValLeu  
 LeuLysAsnAsnLeuThrAlaCysIleAlaAspPheGlyLeuAlaLeuLysPheGluAla

Figure 6

GlyLysSerAlaGlyAspThrHisGlyGlnValGlyThrArgArgTyrMetAlaProGlu  
ValLeuGluGlyAlaIleAsnPheGlnArgAspAlaPheLeuArgIleAspMetTyrAla  
MetGlyLeuValLeuTrpGluLeuAlaSerArgCysThrAlaAlaAspGlyProValAsp  
GluTyrMetLeuProPheGluGluGluIleGlyGlnHisProSerLeuGluAspMetGln  
GluValValValHisLysLysLysArgProValLeuArgAspTyrTrpGlnLysHisAla  
GlyMetAlaMetLeuCysGluThrIleGluGluCysTrpAspHisAspAlaGluAlaArg  
LeuSerAlaGlyCysValGlyGluArgIleThrGlnMetGlnArgLeuThrAsnIleIle  
ThrThrGluAspIleValThrValValThrMetValThrAsnValAspPheProProLys  
GluSerSerLeu\*\*\*

Figure 7



181	CGAGCTTTCTGCTTTTCTCTGG
91	ATAGATGCTAAAGGATTTCCATGGATATACATGGATATTCCTTTATCTGGCATMAATCTAGAGCTCTCCATGGGACAAACTGTTTTC
1	TCCTGGATTCCTTCTTAAATATCTAGCTCATTAAGGGCTTGTGATTTGATTTAGTAAAGAGGACAGAAAGGAAAGGAAAGAAAG
90	ATGGGAGCTGCTACCAAGGCTGGCTTTGCGAGTCTTTCTTTATCTCTGTTTCTCTACCGAGCATTCGATCTCTGAGGCTGCGAAACCAAAAGAA
	<u>HHGAAATIKLAFAVFLILSSSSAGSILGRSETK E</u>
180	TCGATCTACTACAAATCCAACTGGGACAGGACAGCAAAATCCAAAGGACGGAGATCTGCTATTCGGGATATATCATAAAGGAAAGGAC
	<u>CIYYNANHWEKDKTNSHNGTIEICYG D H D K R K H</u>
270	TCCTTTGCAACTTGGAAAGAACTTTTGGGCTCCATAGAAATGTTAAAGAAAGGCTGCTGTTGCAAGATATCAACTGCTATTAAGAAAGGAC
	<u>CFATWHKHISGSIEIVKQGCWLCDINCYHKS</u>
360	AAATGCAACAGCAAAAGGATAGTGCAGATCTGTTTCTGTTCTGGGAAGGAAACTATTCGAAATGAAAGTTCTTACCATTCACAGAG
	<u>KCTEKXDSPPDVFFCCCEGHYCHIEK F Y H S P E</u>
450	ATGGAGGTGCACAGCCGACCTEAAATCTGTGCAACTAAAGCTCTTTATTCACACACTCTGCTTACTCACTGCTGCTCTATCTGTC
	<u>HEVTPQPTSNPVTTTKPLFNITLLYSLVPIHV</u>
540	GTTCAGCTGATTCCTCTCTCTGTTTGGATGTACCGACATCACAGGCTGCGCTACCCGAGTGTGGTTCCAAAGGAGGACCCAGGC
	<u>VAVJVLFSFWHYRHHKLALAYPPVVLVPTODPG</u>
630	CCCTCTCTGCTCTCTCTCTGCTGCTTAAAGCGTTTCAGCTATTCGAGGTAAAGCAAGGAGGCTTTTGGCTGGTCTGCAAGGCT
	<u>FPPPSPLLGLCLKPLQLLEVKARCGRF GCVWK A</u>
720	CAGTTATTAAATGAAGCTGAGCTGTGCAAGTATTCCTCTGTACAGATAAAGCTGTTTCGCAAGGAGTATCACTACAGGCTGCTCT
	<u>QLL L H E I V A V K I F P V O D K L S W O N E Y E I Y S L P</u>
810	CGGATGAAGCATAGAAATATCTGCTACTTCATTCGGCGGAAAGCTGGACAAAGCTTCACAGAGATCTGTTGTTAATACCTGCTTTC
	<u>G H K H E N I L Y F I G A E K R G T N L D T D L W L I T A F</u>
900	CAGCAAAAGGCTCTCTGACAGACTATGTCAAAGCCAAAGCTGTGCTTTCGAAATGAGCTTTCTGCTCATGCTGTCAGCAATATGCGAGAGCT
	<u>H E K G S L T D Y L K A H V V S W N E L C L I A E T H A R G</u>
990	TTATCTACCTCCATGAAATATTCGAGGACTCAAGATGCAACAGGCTGCTGCTAGGACATAGGATATTAAGAGCAAAATGCTCTA
	<u>SHLHEDOIPLGLKDGHKPAVAH R D I KSKNV L</u>

40

[illegible]